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**CONTROL OF  
NUTRITIONAL ANAEMIA  
WITH SPECIAL REFERENCE TO  
IRON DEFICIENCY**

**Report of an IAEA/USAID/WHO Joint Meeting**

WORLD HEALTH ORGANIZATION

GENEVA

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# CONTENTS

	Page
1. Introduction . . . . .	5
2. Deleterious effects of anaemia and of nutritional iron and folate deficiency	6
2.1 Pregnancy . . . . .	6
2.2 Anaemia and work capacity. . . . .	6
2.3 Resistance to infection . . . . .	9
3. Recent scientific advances in the field of iron and folate nutrition . . . . .	10
3.1 Iron balance and its assessment . . . . .	10
3.2 Techniques for measuring absorption of iron from whole diets. . . . .	14
3.3 Folate balance and its assessment . . . . .	16
3.4 Folate content of the diet and its absorption . . . . .	17
3.5 Prevalence of folate deficiency anaemia . . . . .	18
4. Supplementation . . . . .	18
4.1 Therapeutic supplementation . . . . .	18
4.2 Prophylactic supplementation . . . . .	23
5. Fortification . . . . .	25
5.1 Iron . . . . .	25
5.1.1 Forms of iron for fortification . . . . .	25
5.1.2 Vehicles for iron fortification. . . . .	26
5.1.3 Absorption promoters. . . . .	28
5.1.4 Predicting the effectiveness of fortification . . . . .	28
5.2 Folate . . . . .	29
6. A scheme of action for combating nutritional anaemia . . . . .	31
6.1 Definition of the problem. . . . .	31
6.1.1 Determining the status of the population . . . . .	31
6.1.2 Setting the goals . . . . .	31
6.2 Areas with a high prevalence of anaemia . . . . .	34
6.2.1 Pilot therapeutic supplementation trial . . . . .	35
6.2.2 Field therapeutic supplementation trials . . . . .	39
6.2.3 National therapeutic supplementation programme . . . . .	41
6.3 Areas with a moderate prevalence of anaemia . . . . .	41
6.3.1 Pilot prophylactic supplementation trial . . . . .	42
6.3.2 Fortification or supplementation? . . . . .	42
6.3.3 Choice of additive and vehicle for fortification; simulated fortification trial . . . . .	43
6.3.4 Fortification trial . . . . .	44
6.3.5 National fortification programme . . . . .	44
6.3.6 Field prophylactic supplementation trial . . . . .	44
6.3.7 National prophylactic supplementation programme . . . . .	45
7. Recommendations . . . . .	45
7.1 Methodology . . . . .	45

	Page
7.2 Detrimental effects of nutritional anaemia and nutritional deficiencies	46
7.3 Availability of iron from different diets . . . . .	46
7.4 Prevalence of nutritional anaemia and deficiency of haematopoietic nutrients . . . . .	47
7.5 Supplementation . . . . .	47
7.6 Fortification with iron . . . . .	48
7.7 Fortification with folate . . . . .	49
7.8 Education and training . . . . .	49
7.9 International cooperation . . . . .	49
Annex 1. List of participants . . . . .	50
Annex 2. The absorption of iron from whole meals of different types as measured by radioactive iron studies . . . . .	52
Annex 3. Iron compounds for use in iron fortification . . . . .	55
References . . . . .	61

# CONTROL OF NUTRITIONAL ANAEMIA WITH SPECIAL REFERENCE TO IRON DEFICIENCY

## Report of an IAEA/USAID/WHO Joint Meeting

A meeting of experts on nutritional anaemia jointly sponsored by the International Atomic Energy Agency, the Agency for International Development (United States of America), and the World Health Organization was held in Geneva from 28 October to 1 November 1974.

The meeting was opened by Dr W. H. Chang, Assistant Director-General, on behalf of the Director-General.

### 1. INTRODUCTION

Since 1958 WHO has been interested in the problems of nutritional anaemia (1, 2) and has sponsored investigations in a number of countries. These and other recent studies have disclosed a very high prevalence of nutritional anaemia and deficiency of haematopoietic nutrients in people of all age groups, but especially among pregnant women and young children. Although nutritional anaemia is a worldwide problem its prevalence is highest in developing countries (3-19). These studies have also provided valuable information on the etiology of anaemia. It has become clear that iron deficiency is by far the commonest nutritional disorder and the commonest cause of anaemia. The second most common cause of nutritional anaemia is folate<sup>a</sup> deficiency (20-22). Other nutritional deficiencies that play a less important role in the pathogenesis of anaemia are vitamin B<sub>12</sub> deficiency (23, 24) and possibly protein deficiency (25, 26).

Simultaneously with the accumulation of data on prevalence have come an increased understanding of the detrimental effects of anaemia, greater knowledge of other metabolic disorders produced by these nutritional deficiencies, and developments in laboratory techniques for the investigation, diagnosis, and quantification of deficiency states.

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<sup>a</sup> In this report the terms folate and folic acid are used more or less synonymously.

Unfortunately, these scientific advances have so far had little impact on the control of nutritional anaemia, which remains a major public health problem in many parts of the world.

In view of these considerations, the meeting began with a review of some of the available information on the effects of anaemia and recent technological advances in the field of iron and folate nutrition. Attention was then focused on ways and means of combating nutritional anaemia through dietary supplementation and fortification.

## **2. DELETERIOUS EFFECTS OF ANAEMIA AND OF NUTRITIONAL IRON AND FOLATE DEFICIENCY**

### **2.1 Pregnancy**

Severe anaemia in pregnant women increases maternal morbidity and mortality and involves a higher risk for the fetus (27, 28). The deleterious effects of milder forms of anaemia are not well defined, although some studies have suggested a correlation between maternal haemoglobin concentration and fetal birth weight (16). The evidence linking folate deficiency with a possible increase in fetal abnormalities is controversial (29).

### **2.2 Anaemia and work capacity**

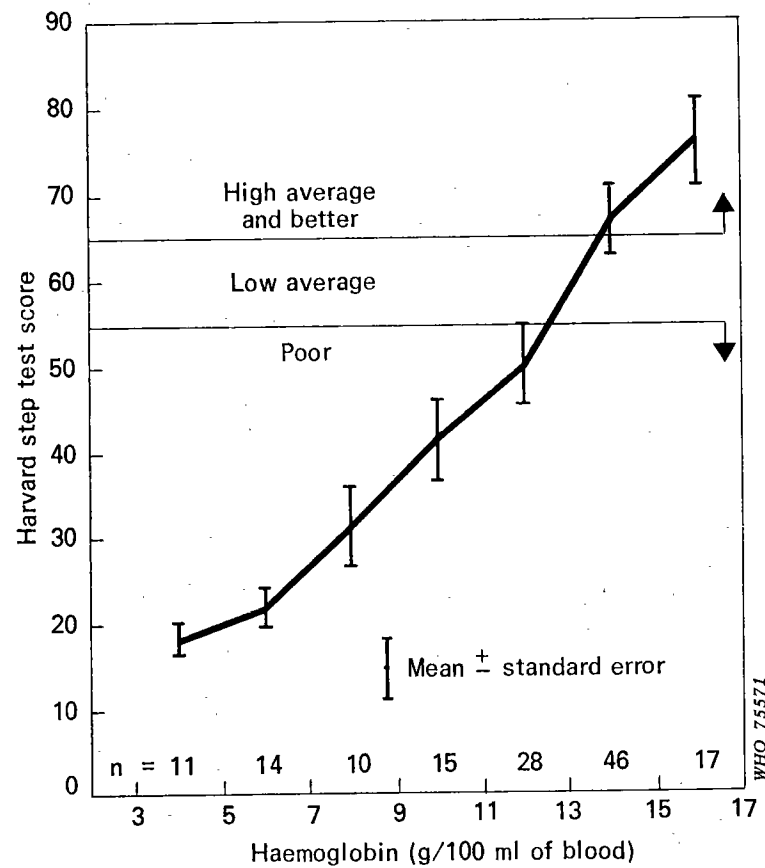
Physical work capacity can be defined as the potential of an individual to engage in activities involving muscle action. Such activities range from strenuous exercise of short duration to mild exercise of long duration, and make use of different mechanisms of physiological adaptation. Individual performance in acute strenuous exercise leading to near-exhaustion (maximum exercise) depends mostly on cardio-respiratory reserve, oxygen delivery, and metabolic adaptation (such as comes with physical training). The body size and composition (lean body mass) of the individual dictate in turn the maximum oxygen consumption, cardiac output, total circulating haemoglobin, and maximum ventilatory rate. All of these variables must be considered when determining the maximum workload that an individual can sustain without becoming exhausted (30). On the other hand, mild exercise of long duration requires continuous energy supply to the muscles and an adequate energy reserve.

A reduction in haemoglobin concentration decreases the oxygen-carrying capacity of the blood, which may reduce oxygen delivery to the tissues during exercise. The more severe the anaemia, the greater the reduction in near-maximum work performance. Physical incapacity supervenes when tissue oxygen demands cannot be met. The critical haemoglobin level (i.e., the level that imposes a limitation on physical activity and work output) varies depending on the severity of the physical effort required and on whether other limiting factors are present. It is possible that the limiting influence of haemoglobin concentration on near-maximum work capacity may be clearly demonstrable only when other limiting factors are absent.

Evidence has been available for a number of years indicating that severe anaemia impairs near-maximum work capacity (31-38). The extent to which work capacity is impaired by mild or moderate anaemia is less easily measured. Studies in Burma failed to demonstrate any difference in physical work capacity in subjects with haemoglobin deficits of up to 2.6 g/100 ml of blood (Yin Thu, Mya Tu & Aung Than Batu, unpublished observations, 1974). However, other recent studies both in experimental animals (39, 40) and in man (41-44) have shown that even small reductions in haemoglobin may result in decreased performance in maximum or near-maximum exercise. Viteri and Torun (30, 45, 46) reported that in sugarcane cutters there was a direct relationship between packed cell volume or haemoglobin concentration and the score on the Harvard step test (47), which measures cardiorespiratory reserve with near-maximum exercise (Fig. 1). When these agricultural workers received iron supplementation of 100 mg/day for 6 months, both their haemoglobin concentration and their score increased in parallel fashion, while a control group given placebo tablets showed no change in either haemoglobin or exercise performance. The beneficial effects of iron treatment became evident within 1 month after its initiation, reached a maximum at 2 months, and remained stable thereafter. In this study, differences in haemoglobin concentration of only about 1.5 g/100 ml of blood were accompanied by significant differences in physical performance.

Anaemia reduces the capacity to perform energy-demanding tasks by imposing a ceiling on oxygen transport to the tissues. From the data available, and from theoretical considerations on maximum oxygen delivery to tissues, one can compute the maximum predicted workload that can be sustained by individuals with various haemoglobin concentrations and maximum cardiac outputs (Table 1). The relevance of these workload data to the actual working conditions and work output of

FIG. 1. HARVARD STEP TEST SCORE IN GUATEMALAN AGRICULTURAL LABOURERS WITH DIFFERENT HAEMOGLOBIN CONCENTRATIONS \*



\* Reproduced with permission from VITERI, F. E. & TORÚN, B. Anaemia and physical work capacity. *Clin. Haematol.*, 3: 609-626 (1974).

populations engaged in hard physical labour can be predicted from the energy cost of various agricultural tasks (46, 48). However, the physical effort required in most occupations seldom approaches near-maximum exercise, and it is not known whether milder degrees of anaemia affect such non-maximum work performance. Well planned studies to explore this question are urgently needed.



TABLE 1. MAXIMUM PREDICTED WORKLOAD THAT CAN BE SUSTAINED BY INDIVIDUALS WITH VARIOUS HAEMOGLOBIN CONCENTRATIONS AND 3 DIFFERENT MAXIMUM CARDIAC OUTPUTS (30)

Haemoglobin concentration (g/100 ml of blood)	Maximum predicted workload <sup>a</sup> with maximum cardiac output of :		
	15 litres/min	20 litres/min	25 litres/min
4	3.2 (13.4)	4.2 (17.6)	5.3 (22.2)
6	4.8 (20.1)	6.4 (26.8)	8.0 (33.5)
8	6.4 (26.8)	8.5 (35.6)	10.6 (44.4)
10	8.0 (33.5)	10.6 (44.4)	13.2 (55.2)
12	9.6 (40.2)	12.7 (53.1)	15.9 (66.5)
14	11.1 (46.4)	14.8 (61.9)	18.5 (77.4)
16	12.7 (53.1)	17.0 (71.1)	21.2 (88.7)

<sup>a</sup> Workload is expressed here in both kilocalories and kilojoules per minute, the latter being shown in parentheses. Conversion factors are : 1 kcal = 4.184 kJ ; 1 J = 0.239 cal.

### 2.3 Resistance to infection

There is increasing evidence that anaemia and iron deficiency may play a role in the ability of the individual to resist infection.

The body's defence against microorganisms depends to a large extent upon the phagocytic activity of the white cells that ingest and destroy invading bacteria, and upon a complex series of events leading to the development of cellular and humoral immunity. For example, granulocytes kill bacteria through the activity of their cytoplasmic enzyme myeloperoxidase coupled with a hydrogen peroxide generating system (49). This enzyme is moderately reduced in iron deficiency anaemia and returns to normal following iron therapy (50-52). It is possible that such a reduction in myeloperoxidase could affect host resistance, as has been suggested by studies on iron-deficient weanling rats exposed to *Salmonella typhimurium* (53).

Iron deficiency may also impair the immune response. For example, iron-deficient weanling rats showed a significantly impaired humoral antibody response after administration of tetanus toxoid (54). In another study, patients with iron deficiency were found to have an impairment of lymphocyte transformation and migration inhibition factor production (55). There is also some evidence in man that iron deficiency reduces the capacity of the epithelium of the skin and mucosa to resist colonization by organisms such as *Candida* (56).

Epidemiological studies that attempt to relate infection rates to the prevalence of anaemia are difficult to interpret but there is some evidence

that iron deficiency anaemia may make children more prone to respiratory infections (57, 58). On the other hand, in a recent study from East Africa, an increase in malarial attacks was reported when patients with iron deficiency anaemia were treated with iron (50). However, this may have been due to the increase in reticulocytes following iron therapy rather than to a direct potentiating effect of the administered iron on the growth of the parasites.

### **3. RECENT SCIENTIFIC ADVANCES IN THE FIELD OF IRON AND FOLATE NUTRITION**

There has been a steady expansion of knowledge in the field of iron and folate nutrition. Recent scientific advances in the following areas, which have a potential direct application to the evaluation and control of nutritional anaemia, were reviewed by the meeting :

- iron balance and its assessment
- techniques for measuring the absorption of iron from whole diets
- folate balance and its assessment
- folate content of the diet and dietary folate absorption
- prevalence of folate deficiency anaemia.

#### **3.1 Iron balance and its assessment**

Iron balance in man depends on three factors — the iron requirement for production of haemoglobin, the iron losses from physiological and pathological processes, and the amount of iron ingested and absorbed from the intestine. Iron requirements are greatest when there is rapid expansion of tissue and red cell mass, such as occurs in infancy, childhood, and pregnancy. Iron is lost mainly from the uterus (in women of childbearing age) and the gastrointestinal tract. Menstrual iron losses have been measured in women from several countries, including Burma (60), Canada (61), India (62), and Sweden (63), and appear to be similar in all, with about 10% of women having a loss of more than 1.4 mg/day. Iron losses are lower in women taking oral contraceptives and higher in those using intrauterine contraceptive devices. Physiological losses from all other routes are of the order of 14 µg/day/kg of body weight (64). Studies carried out by chemical balance had previously suggested that in hot climates greater amounts of iron might be lost in sweat (65, 66),

but more recent long-term studies with  $^{55}\text{Fe}$ -labelled iron have shown that iron losses are not increased in subjects working in hot, humid climates (67). The high prevalence of iron deficiency in many tropical countries therefore cannot be explained on this basis.

Pathological iron losses may occur in menorrhagia and in diseases of the gastrointestinal tract. The most important cause of pathological iron loss is hookworm infestation (68), which affects a large proportion of the world's population. Blood loss with *Necator americanus* infestation is about 0.03 ml/day per worm, or about 2.0 ml with a load of 1 000 eggs/g of faeces. After the partial reabsorption of haem iron is taken into account this latter amount represents a daily loss of about 0.8 mg of iron. With *Ancylostoma duodenale* the daily blood loss is about 0.2 ml per worm, or with a load of 1 000 eggs/g of faeces about 4.5 ml, representing an iron loss of about 1.8 mg (2).

The amount of iron absorbed depends on the amount of iron ingested and its absorbability. This is discussed in detail in section 3.2. Diseases of the intestine such as coeliac disease, tropical sprue, and the widely prevalent tropical enteropathy (69) may also decrease iron absorption.

Iron deficiency is most likely to occur when iron requirements are greatest, i.e., during infancy, childhood, the reproductive age in women, and pregnancy. In developing countries, apart from increased requirements the commonest causes of iron deficiency are poor availability of dietary iron and increased iron losses due to parasitic infestation, especially hookworm. In developed countries iron deficiency is less common but when it occurs it may be due to inadequate dietary iron intake associated with reduced energy intake, or to the presence of some pathological state interfering with iron absorption or producing increased iron losses.

Present-day laboratory procedures for assessing iron status are best understood if they are related to the 3 stages of iron deficiency.

The first stage of iron deficiency is characterized by decreased storage iron without any other detectable abnormalities. In experimental studies, the size of the iron stores can be measured in volunteers by performing phlebotomies until there is evidence of iron deficient erythropoiesis (70). The size of the mobilizable iron stores is then calculated from the amount of haemoglobin removed. The results obtained with this approach by different workers have recently been reviewed (71). A more convenient but less quantitative method of assessing iron stores is the histological examination of the amounts of stainable iron present in the reticuloendothelial cells of the bone marrow (72). This method has been widely applied in clinical studies. A third approach is the

measurement of iron absorption. As stores decrease, there is a concomitant increase in iron absorption from the gut (73). However, measurement of iron absorption is not a practical method of assessing the iron status of large groups of subjects.

The second stage of iron deficiency begins after iron stores are exhausted. With further iron depletion there is a restriction of haemoglobin synthesis; the percentage saturation of transferrin falls from a normal value of about 30% to less than 15% (74) and the concentration of protoporphyrin in red cells rises to more than 70  $\mu\text{g}/100\text{ ml}$  (75).

The third stage, that of overt iron deficiency, is reached when the impaired haemoglobin synthesis results in a measurable decrease in the concentration of circulating haemoglobin. Initially, the red cells are normocytic and normochromic but ultimately they exhibit the classical morphological features associated with iron deficiency anaemia.

Although it is usually anaemia that alerts the physician to the presence of iron deficiency in the individual patient, the use of haemoglobin concentration or the haematocrit as an index of iron deficiency in population surveys has limitations. Where there is a high prevalence of anaemia, measurements of haemoglobin may suffice. However, where iron deficiency is less severe there will be a greater overlap between the distribution curves for normal and iron-deficient subjects, introducing a significant error in the identification of those with the deficiency. In other words, the value of haemoglobin as an indicator of iron deficiency decreases as the severity of the deficiency in the population decreases. The emphasis has consequently shifted over the last 10 years to devising more precise methods for identifying iron deficiency (2, 76). Histological examination of bone marrow or liver specimens and studies of iron absorption both provide good indices of the size of iron stores but cannot be applied on a wide scale; on the other hand, measurement of the percentage saturation of transferrin, which reflects the adequacy of iron supply to the marrow, is a labile measurement and a less specific index. The current need, therefore, is for some practical means of assessing the size of body iron stores that can be applied to population surveys. Recently, immunoradiometric methods have been developed for the measurement of serum ferritin (77, 78) and several studies suggest that such estimations may provide a sensitive index of storage iron status. Since this discovery promises to have widespread implications the evidence relating to its significance is reviewed below in some detail.

In normal subjects in the United Kingdom the mean value of serum ferritin for males was 69.2  $\mu\text{g}/\text{litre}$  (standard deviation 5.2) and that for females was 34.8  $\mu\text{g}/\text{litre}$  (standard deviation 5.1) (79). In a population

survey of 346 normal individuals in the USA a skewed distribution of ferritin values was found, with a geometric mean of 59 µg/litre (95% range: 12–300 µg/litre) and a mean value for adult males of 94 µg/litre and for adult females of 34 µg/litre (80). The evidence that there is a close relationship between serum ferritin concentration and size of body iron stores includes the following:

- In normal subjects there is a close correlation between serum ferritin concentration and the size of body iron stores as measured by repeated phlebotomies (71, 79).
- The known difference between iron stores in men and women is reflected by a proportionate sex difference in the concentration of serum ferritin (77–80).
- There is a significant correlation between serum ferritin and iron stores as judged by the histological assessment of bone marrow haemosiderin (81).
- There is an inverse correlation between serum ferritin and iron absorption in normal subjects (80).
- In iron deficiency states the serum ferritin is very low (mean value 4 µg/litre) while with iron overload it is very high (> 1 000 µg/litre) (79, 81).
- When subjects with hypoplastic anaemia are given repeated blood transfusions the serum ferritin rises progressively; similarly, a predictable fall occurs in patients with iron overload subjected to repeated phlebotomies (79, 81). These results suggest that a serum ferritin concentration of 1 µg/litre is equivalent to about 10 mg of storage iron.

While these findings underline the potential value of serum ferritin estimations as an index of storage iron status, there are certain conditions under which ferritin levels are not directly proportional to body iron stores; these include infections, some liver diseases, haemolytic states, and conditions associated with highly ineffective erythropoiesis (81). Studies correlating ferritin levels with C-reactive protein may further clarify the effect of infection. Even in these conditions, however, it should be stressed that the size of the iron stores remains the major determinant of the serum ferritin concentration.

From the evidence already available there appear to be good grounds for believing that serum ferritin measurement may prove to be the single most effective tool for evaluating the iron status of different population groups and especially those in which anaemia is not prevalent. It will

also provide a sensitive means of assessing the effects of any programmes aimed at improving the iron nutrition of particular population groups. In addition, serum ferritin measurements may enable individuals with microcytic anaemia secondary to infection or haemoglobinopathy to be differentiated from those with iron deficiency.

### **3.2 Techniques for measuring absorption of iron from whole diets**

The absorption of iron from individual foods has been considered in previous WHO publications (2, 64, 76). Studies with biosynthetically radioactive labelled foods have shown that in normal subjects there is a variation in iron absorption from less than 1% with some vegetable foods to 10–25% with meat (76, 82). The small proportion of iron absorbed from vegetable foods suggests that they may contain inhibitors of iron absorption. The inhibitory effect of phytates and phosphates has been suspected for many years. Detailed studies of wheat bran indicate that in this material it is the phytate rather than the phosphate content that has the inhibitory effect on iron absorption (83). Other foods that inhibit iron absorption are milk, eggs (84), and tea. The effect of tea is particularly striking; iron absorption from a meal containing biosynthetically labelled rice decreased from 12% to 2% when tea was taken with the meal (85).

There are also factors in the diet that increase non-haem iron absorption, such as red meat, fish, chicken, and liver (86–89). The mechanism of this enhancing effect is not fully understood, but the effect is greater when phytic acid is present, which suggests that the meat is acting as an “anti-inhibitor” (L. Hallberg, unpublished observations, 1974).

Ascorbic acid also enhances the absorption of non-haem iron (76), as do fruits in proportion to their ascorbate content (84, 90). More recent studies have confirmed this iron absorption-enhancing effect of ascorbate when it is added to a variety of foodstuffs (91–93). It has also been shown that ascorbic acid increases iron absorption from iron-enriched foods (94).

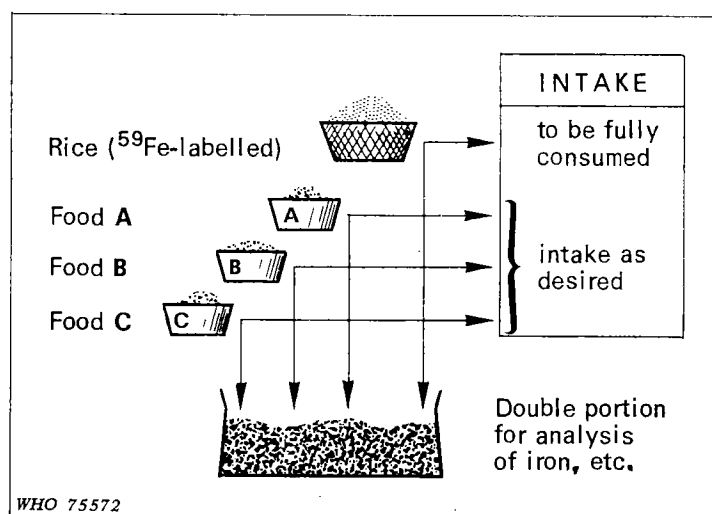
Because of the multiplicity of factors that may affect iron absorption, it is not possible to make valid estimates of total iron absorption from a composite meal even of known iron content. Actual measurements of iron absorption from typical meals are necessary.

Dietary iron absorption takes place from two independent pools — a haem iron pool and a non-haem iron pool (95, 96). These two pools can be separately labelled with two different radioactive isotopes of iron and the iron absorption from both pools can then be measured with con-

siderable accuracy. Ferritin, haemosiderin, and contaminating iron from some soils (L. Hallberg, unpublished observations, 1974) do not fully exchange with an inorganic extrinsic tracer (97). Since ferritin and haemosiderin form only a small part of the non-haem iron in most meals, the errors introduced by ignoring these when one measures the iron absorption from composite meals will be negligible. However, the influence of contaminating soil iron may be more significant and needs further study.

In diets where the haem iron pool is small or absent (as in many developing countries) the procedure for measuring total dietary iron absorption can be considerably simplified by using only one radioactive isotope of iron to label the non-haem iron pool. This "extrinsic tag" when carefully mixed with a homogenized meal or a vegetable food before cooking gives accurate and reproducible results (98-103). More recently, a simpler technique has been proposed (L. Hallberg, unpublished observations, 1974) whereby the labelled iron is added to the main bulky component of the meal after cooking (e.g., rice, mashed potatoes, or bread). This enables the test to be utilized in field studies, the duplicate meal technique being used to measure the total dietary iron. The subjects thus have a full choice of foods except for the one radioiron-labelled component, which must be fully consumed (Fig. 2).

FIG. 2. DIAGRAMMATIC REPRESENTATION OF A FIELD METHOD FOR STUDYING THE ABSORPTION OF IRON FROM THE WHOLE DIET WITH THE USE OF AN EXTRINSIC LABEL OF RADIOACTIVE IRON



The amount of iron absorbed from a given meal will depend to a large extent on the iron status of the individual (73). In order, therefore, to be able to compare validly the results of absorption from different meals in different individuals, it is necessary to compare absorption from the meal with absorption from a reference dose of an easily absorbable iron salt. A dose of 3 mg of iron as freshly prepared iron(II)<sup>a</sup> ascorbate administered to the subject in a fasting state is recommended as a standard. If such a reference dose is used routinely it will permit absorption studies to be compared in different population groups by comparing the regression functions of food iron absorption on absorption of the reference dose. Some recent data on iron absorption from different types of meals are given in Annex 2 to this report (pages 52-54).

### 3.3 Folate balance and its assessment

The folate balance depends on the folate requirements for deoxyribonucleic acid synthesis and other biochemical functions, the amount of folate lost from the body, and the amount ingested and absorbed from the diet.

Folate requirements are greatest in conditions where there is rapid cell multiplication, such as during growth in young children and during pregnancy. Requirements are also increased in certain disease states such as infections and haemolytic anaemias. Small losses of folate occur in the urine and from the gastrointestinal tract but these are not nutritionally important except in some gastrointestinal diseases (104).

The amount of folate absorbed depends on the amount in the diet, the amount lost from cooking and food processing, and the integrity of the intestine. This is discussed in detail in section 3.4. As with iron, disease of the gastrointestinal tract may decrease folate absorption. Folate metabolism may also be adversely affected by the ingestion of alcohol and various drugs, such as anticonvulsants, contraceptives, and antimalarials (105, 106).

A negative folate balance is most often the combined result of deficient dietary intake and increased demands. The laboratory diagnosis of folate deficiency is based on the measurement of serum and red cell folate concentrations, usually by microbiological assay. This has been considered in detail in previous WHO publications (2, 76). Unfortunately, because of the complexity of microbiological assays there is still

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<sup>a</sup> Following standard chemical nomenclature, "iron(II)" is used here for "ferrous" and "iron(III)" for "ferric".



considerable interlaboratory variation in these measurements, and better international standardization of folate assays is urgently needed. More recently, radioisotopic dilution techniques have been suggested (107) but these have not yet gained wide acceptance.

When there is a negative folate balance the folate concentration falls first in the serum and some weeks later in the red cells (108). A serum concentration of less than 3 ng/ml and a red cell concentration of less than 100 ng/ml of red cells may be taken as indicative of deficiency (76). However, folate concentration correlates poorly with the presence or severity of megaloblastic anaemia (109). Moreover, individuals may have lowered serum and red cell concentrations without any obvious impairment of health (108, 110) apart from a greater liability to develop haematological manifestations of folate deficiency.

#### **3.4 Folate content of the diet and its absorption**

Most of the available data on the folate content of the diet were obtained before the importance of using ascorbate and conjugase in the assay of food folate was established (76). Ascorbate is necessary to prevent the loss of heat-labile forms during the processes of extraction and assay. Since most folates exist in food in the form of polyglutamates that are not growth promoters for the assay organisms, conjugase is needed to split the polyglutamates to the simpler forms to which the assay organisms will respond. Even when ascorbic acid and conjugase have been incorporated in the assay procedure, however, there are quite marked differences in the estimates of food folates from different laboratories. The cause of these variations is not clear. They may be due to genuine differences in the folate content of the foods assayed in different parts of the world, or to differences in such factors as water content or the degree of ripeness of vegetable products. Alternatively, they may result from differences in the extraction and assay procedures of different laboratories, including the type of conjugase used. It is also possible that nonspecific binding of folate to cellulose fibre may variably affect the amount of assayable folate (111, 112).

It is well established that the cooking process may cause a considerable reduction in folate activity. This effect is particularly marked when food is boiled for prolonged periods (113–115). The conditions under which food is stored prior to consumption may also be important. Thus, liver stored at room temperature loses its folate activity rapidly but the loss is greatly retarded by storage at +4°C or –20°C (G. Izaak, unpublished observations, 1974).

Folic acid is maximally absorbed in the duodenum and upper jejunum, although absorption can also occur from the lower small intestine (116). There is good evidence, from studies with isotopically labelled folates, that folate polyglutamates are deconjugated to monoglutamate forms during the process of absorption (117, 118). Evidence regarding the relative availability of food folate as compared with folic acid (pteroyl-monoglutamic acid) is conflicting. A number of studies indicate that polyglutamates are less available than the corresponding amounts of monoglutamate (76, 119-121). However, many of these studies were done either with yeast polyglutamates, which may contain a conjugase inhibitor, or with synthetic polyglutamates. Recent studies in which the rise in serum folate concentration was measured after the ingestion of liver, meat, lettuce, broccoli, cheese, eggs, and spinach yielded curves of serum folate concentration rather similar to those found after the ingestion of amounts of folic acid comparable to the total folate content of the food (measured after treatment with conjugase). The sole exception among the foodstuffs tested was beans, which produced no detectable elevation in serum folate activity (G. Izaak et al., unpublished observations, 1974). Although serum folate concentration is only an indirect indicator of absorption, these findings suggest that dietary folate is liberated and absorbed with reasonable efficiency by the gastrointestinal tract of normal subjects.

### **3.5 Prevalence of folate deficiency anaemia**

Although iron deficiency is the commonest cause of nutritional anaemia, folate deficiency is also prevalent in many population groups even in developed countries (4, 9, 20, 120, 122-125).

Since iron deficiency may mask the presence of concurrent folate deficiency (126) the true prevalence of anaemia due to folate deficiency can best be established by therapeutic supplementation trials (18, 21, 22, 127). More studies of this type are needed in different parts of the world.

## **4. SUPPLEMENTATION**

### **4.1 Therapeutic supplementation**

Where there is a high prevalence of anaemia, especially anaemia due to iron deficiency, the only way of improving the situation within a reasonable period of time is to provide therapeutic supplements. Supplementation is the only possible approach in particular for pregnant women, in whom it is necessary to raise the haemoglobin concentration to a satisfactory level before delivery.

The results of two recent supplementation trials in pregnant women were considered at the meeting. In a trial in Israel (127) anaemic women (haemoglobin concentration  $< 10$  g/100 ml of blood) were divided into 3 groups and given daily medication from the second trimester of pregnancy until term. One group received 100 mg of iron and 300  $\mu$ g of folate, the second 100 mg of iron only, and the third 300  $\mu$ g of folic acid alone. Of the women receiving both iron and folate, 90% showed a rise in haemoglobin, whereas only 26% of those receiving either iron or folate alone showed such a rise, which indicated the superiority of iron and folate together. Based on the results of this trial, a programme was implemented in which 100 mg of iron and 300  $\mu$ g of folate were given to all pregnant women in a selected community from the second trimester until term. This supplementation resulted in the reduction of the prevalence of anaemia of pregnancy (haemoglobin concentration  $< 10$  g/100 ml) from over 50% to below 6%.

In India, previous studies had shown a very high prevalence of nutritional anaemia of pregnancy, apparently due to iron and folate deficiency (2, 9, 16, 21). Collaborative WHO supplementation trials were therefore initiated (18). Since the design and results of these studies are considered important for the planning of action to measure the prevalence of anaemia and devise methods for its control, they are described here in some detail.

Pregnant women with no clinical evidence of any associated disease were admitted to the trial at  $22 \pm 2$  weeks of gestation and were randomly allocated to two streams, A and B, there being 2 women in stream A for every 5 in stream B. The women in stream A were given placebo tablets and placebo injections, while those in stream B received 5 mg of folic acid daily 6 days a week and 100 mg of vitamin B<sub>12</sub> (cyanocobalamin) by intramuscular injection fortnightly for 4 weeks. The aim of the treatment given to women in stream B was to correct any significant pre-existing folate and vitamin B<sub>12</sub> deficiency that might vitiate the subsequent stratification procedure and thus interfere with the final interpretation of the response to different doses of iron. In practice, this step proved to be unnecessary.

To ensure that the treatment groups would have a more even composition in terms of initial haemoglobin concentration a stratification procedure was used. At  $26 \pm 2$  weeks of gestation the women were divided into 3 strata depending on their initial haemoglobin values (5.0–7.9 g/100 ml, 8.0–10.9 g/100 ml, and  $\geq 11.0$  g/100 ml, respectively). A set of random tables was then prepared for each stratum and utilized for randomly allocating the women in that stratum to the various

TABLE 2. THERAPY GIVEN TO DIFFERENT GROUPS OF PREGNANT WOMEN IN SUPPLEMENTATION TRIAL IN INDIA (18)

Therapy	Stream A			Stream B				
	Group 0	Group 6	Group 1	Group 2	Group 3	Group 4	Group 5	
Vitamin B <sub>12</sub> <sup>a</sup>	placebo	placebo	100 µg	100 µg	100 µg	100 µg	100 µg	100 µg
Folic acid <sup>b</sup>	placebo	placebo	5 mg	5 mg	5 mg	5 mg	5 mg	5 mg
Iron <sup>b</sup>	—	100 mg	—	30 mg	60 mg	120 mg	240 mg	

<sup>a</sup> Given by Injection fortnightly.<sup>b</sup> Given daily, 6 days a week, in 2 pills each containing half the amount of iron and folic acid shown.

treatment groups. The women in stream A were allocated to either group 0 or group 6 and those in stream B to group 1, 2, 3, 4, or 5. Table 2 shows the supplements given to each group. The injections of vitamin B<sub>12</sub> were given fortnightly and the tablets daily, 6 days a week, for a total of 10–12 weeks, up to week 34–40 of gestation. All supplements were administered under the daily personal supervision of a public health nurse and were given at random times during the day with no fixed relation to meal times.

During the preliminary 4 weeks (22–26 weeks of gestation) the subjects in both streams showed a similar fall in mean haemoglobin concentration, which indicated a lack of response of the women in stream B to folate and vitamin B<sub>12</sub>. In the subsequent 10–12 weeks the mean haemoglobin concentration rose significantly in all the groups given iron but declined further in the groups given no iron. The results clearly demonstrated that iron deficiency was the dominant factor in the causation of anaemia in these women. The women who received folic acid and vitamin B<sub>12</sub> in addition to iron (group 4) had a greater rise in mean haemoglobin concentration than those given an equivalent amount of iron without vitamin B<sub>12</sub> and folic acid (group 6), which suggested that either folic acid or vitamin B<sub>12</sub> deficiency was an important factor in the pathogenesis of their anaemia. Since other studies indicate that vitamin B<sub>12</sub> deficiency plays a very small role in the pathogenesis of pregnancy anaemia in India (16, 21), it is assumed that the better results in group 4 (as compared with group 6) can be attributed to their folate supplementation. However, the design of the trial does not permit confirmation of this assumption.

Further study of the results showed that the rise in haemoglobin was greater in women with a lower initial haemoglobin concentration. The regression lines of final haemoglobin values on initial haemoglobin values for the different supplementation groups were therefore calculated and found to be heterogeneous (Table 3). When the results were analysed for a statistical comparison of regression lines, the differences in slope of the regression lines were found to be highly significant ( $P < 0.001$ ). In view of this finding, separate regression functions for each treatment group were estimated and the expected final haemoglobin concentration was calculated on the basis of the initial haemoglobin value (Table 4). This type of analysis yields more information than a determination of the number of women showing a rise or fall in haemoglobin or of the mean differences between initial and final haemoglobin concentrations, because it takes into account the differences in initial haemoglobin levels in the different groups. Further, such an analysis brings out the differences

TABLE 3. COMPARISON OF REGRESSION LINES: HAEMOGLOBIN VALUES AFTER THERAPY ON INITIAL HAEMOGLOBIN VALUES \*

Group	Source of variation	Degrees of freedom	$\Sigma (X-\bar{X})^2$	$\Sigma (X-\bar{X})(Y-\bar{Y})$	$\Sigma (Y-\bar{Y})^2$	Regression coefficient	Deviations from regression		
							Degrees of freedom	Sum of squares	Mean square
Within treatment groups :									
0	Placebo	69	96.62	92.96	130.85	0.96	68	41.41	0.61
1	B <sub>12</sub> + F.A. <sup>a</sup>	90	144.76	110.39	154.98	0.76	89	70.80	0.80
2	B <sub>12</sub> + F.A. <sup>a</sup> + 30 mg of iron	88	177.59	115.55	140.17	0.65	87	64.99	0.75
3	B <sub>12</sub> + F.A. <sup>a</sup> + 60 mg of iron	90	200.77	106.35	165.53	0.53	89	109.20	1.23
4	B <sub>12</sub> + F.A. <sup>a</sup> + 120 mg of iron	114	214.09	50.67	136.99	0.24	113	125.00	1.11
5	B <sub>12</sub> + F.A. <sup>a</sup> + 240 mg of iron	83	167.92	56.52	105.95	0.34	82	86.93	1.06
6	120 mg of iron alone	106	190.56	133.33	185.74	0.70	105	92.45	0.88
Pooled within treatment groups :		640	1192.31	665.77	1020.21	0.56	633	590.78	0.93
							639	648.45	1.01
							6	57.67	9.61
Difference between slopes :									
Overall comparison between slopes : $F = 10.33$ (d.f. = 6,633) $P < 0.001$									

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<sup>a</sup> Vitamin B<sub>12</sub> and folic acid.

in response to specific supplementation regimens at different levels of initial haemoglobin concentration.

By any analysis, the results were most satisfactory in the groups given 120 or 240 mg of iron together with folate and vitamin B<sub>12</sub>, but even in the group receiving 240 mg of iron with folate and vitamin B<sub>12</sub>, the haemoglobin concentration did not rise above 11 g/100 ml of blood in about 50% of the women. In a subsequent trial parenterally administered iron produced better results than oral iron, which suggested that the oral iron supplements had been relatively poorly absorbed. This poor absorption may have been related either to inhibitory factors in the diet (the pills were not given at any fixed time in relation to meals) or to the intestinal mucosal abnormalities widely prevalent in India (128). The addition of 500 mg of ascorbic acid to the iron supplement did not produce a larger rise in haemoglobin than that produced by iron alone. When 15 g of protein, as calcium caseinate, was given together with 120 mg of oral iron plus folic acid and vitamin B<sub>12</sub>, the response was significantly better than that obtained without the protein supplement. The reasons for this beneficial effect of protein are not clear. Either the protein supplementation enhanced the iron absorption or it caused a direct improvement in erythropoiesis. Further investigation is needed to elucidate the mechanisms of this effect. Interestingly, the prevalence of gastrointestinal disturbances was lower in women who received both iron and folic acid in the same pill than in women treated with iron alone. When ascorbic acid (500 mg) was given along with 120 mg of iron there was a higher prevalence of side effects than with iron alone. A similar observation on the effects of ascorbic acid has been made previously (129).

These supplementation studies show that in populations where nutritional anaemia is prevalent it is possible, through appropriately designed trials, to determine precisely what deficiencies are responsible for it and hence to plan for larger-scale supplementation, as was done in a region of Israel. However, where health services are not well developed, there is a need to carry out operational research into methods of delivering the supplements to larger population groups, with particular emphasis on benefits in relation to costs.

#### **4.2 Prophylactic supplementation**

Where the prevalence and severity of anaemia is lower, more time may be available to correct deficiency states. In many cases food fortification can be used for this purpose (see section 5). However,

TABLE 4. ESTIMATED REGRESSION FUNCTIONS AND EXPECTED MEAN HAEMOGLOBIN CONCENTRATION AFTER 10 WEEKS OF THERAPY DEPENDING ON INITIAL HAEMOGLOBIN CONCENTRATION \*

Group	Treatment	Estimated regression function	Expected (mean) haemoglobin concentration after 10 weeks' therapy				
			6	7	8	9	10
0	Placebo						
1	B <sub>12</sub> + F.A. <sup>a</sup>	$Y = 0.19 + 0.96 X$	5.95	6.91	7.87	8.83	9.79
2	B <sub>12</sub> + F.A. <sup>a</sup> + 30 mg of iron	$Y = 2.08 + 0.76 X$	6.64	7.40	8.16	8.92	9.68
3	B <sub>12</sub> + F.A. <sup>a</sup> + 60 mg of iron	$Y = 4.11 + 0.65 X$	8.01	8.66	9.31	9.96	10.61
4	B <sub>12</sub> + F.A. <sup>a</sup> + 120 mg of iron	$Y = 5.37 + 0.53 X$	8.55	9.08	9.61	10.14	10.67
5	B <sub>12</sub> + F.A. <sup>a</sup> + 240 mg of iron	$Y = 8.16 + 0.28 X$	9.84	10.12	10.40	10.68	10.96
6	120 mg of iron alone	$Y = 8.18 + 0.28 X$	9.86	10.14	10.42	10.70	10.98
		$Y = 3.62 + 0.70 X$	7.82	8.52	9.22	9.92	10.62

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<sup>a</sup> Vitamin B<sub>12</sub> and folic acid.



where fortification is not possible or practicable, the diet may be supplemented with nutrients (in smaller amounts than required as therapy for anaemic populations) to build up body stores and prevent the development of anaemia under conditions of stress. Such prophylactic supplementation is often given, for instance, to premature infants who begin extrauterine life with low iron stores.

## 5. FORTIFICATION

### 5.1 Iron

As discussed in previous WHO publications (2, 64, 76, 130), the amount of available iron in the diet can be increased by fortifying foods with iron. In regions where the amount of available iron contained in traditional diets is inadequate to meet the requirements of a large proportion of the community, fortification could theoretically provide enough additional dietary iron to maintain the majority of the population in iron balance.

Problems related to the form of iron to be added, the vehicle, the product to be fortified, the level of fortification, and the evaluation of its effectiveness were considered at the meeting.

#### 5.1.1 *Forms of iron for fortification*

The type of iron used for fortification must be one that is readily assimilated, does not cause undesirable changes in the vehicle or other food, and is stable under locally prevailing storage conditions. Unfortunately, many iron compounds, especially those most readily absorbed, are highly reactive and produce a variety of undesirable changes in foods to which they are added.

The following chemical properties of iron compounds must be borne in mind :

- iron(II) salts may be oxidized to form yellow, green, or black iron(III) oxides ;
- iron salts may react with phenolic compounds such as tannins and propyl gallate to form blue-black colours ;
- iron compounds may react with sulfur compounds to produce a black colour ;
- iron compounds may increase the activity of oxidative enzymes that cause off-colours, -odours, and -flavours ;
- such compounds may catalyse the development of oxidative reactions.

Also to be taken into consideration are the following physical properties :

- high density products, such as iron powders, may make it difficult to achieve a uniform and stable distribution in powdered foodstuffs ;
- the colour and flavour of the iron compounds themselves may be undesirable and carry through into the food ;
- magnets for removing tramp metal cannot be utilized in food processing when metallic iron products are used for fortification ;
- the solubility characteristics of the iron may not be compatible with the food to be fortified.

Whether and to what extent these chemical and physical properties create problems depends in part on the conditions of processing, storage, and distribution. Many of the chemical and enzymatic reactions mentioned above take place rather slowly or can be retarded by appropriate storage conditions. If the food product is to be consumed within a short time after preparation, it is often possible to use a soluble form of iron that would be completely unsatisfactory in a food requiring lengthy storage under adverse conditions.

At the present time, metallic iron powders (reduced iron), iron(II) sulfate, iron(II) orthophosphate, and iron sodium pyrophosphate are the most commonly used forms of iron for food fortification. Iron(II) sulfate is recognized as the most absorbable of these, in addition to being one of the least expensive iron salts. However, it is also the most chemically reactive of these forms of iron, which makes it difficult to use in many foods. The iron powders, which are relatively insoluble, are in widespread use because of their chemical inertness and low price. Their absorbability varies quite widely, depending on particle size or more probably on surface area per unit weight. Increasing the surface area improves absorbability but also tends to increase chemical reactivity. Although the iron phosphates have been widely used, they are poorly absorbed (131) unless given with an additive such as ascorbic acid (92, 94) and sodium dihydrogen phosphate (132). Information regarding some forms of iron currently being used or considered for use in food fortification can be found in Annex 3, page 55.

#### 5.1.2 *Vehicles for iron fortification*

The vehicle chosen should be one that is already consumed in adequate amounts by the people in need ; one that is available for fortification in

relatively few centres so that quality can be adequately controlled and monitored ; one that is suitable for fortification on a large scale ; and one that results in a product which is stable under extreme conditions of storage and does not alter the palatability of the food.

(a) *Cereals*

Several countries (e.g., Sweden, the United Kingdom, and the USA) already have programmes in operation for the fortification of wheat flour with reduced iron and compounds such as iron carbonyl and iron(II) sulfate. There is, however, no published evidence concerning the nutritional value of these measures (133). Of interest is the recent observation that, when added to bread rolls, some forms of reduced iron are absorbed as well as is iron(II) sulfate (131).

While wheat flour provides a convenient vehicle for iron fortification in developed countries, it is less satisfactory for developing countries where wheat is not centrally milled or where other cereals form the staple foodstuffs. It is also doubtful whether iron can be expected to be adequately absorbed from fortified cereals by people who infrequently eat meat, a known enhancer of iron absorption.

(b) *Salt*

Salt is universally consumed in reasonable quantities by all population groups, but its use as a fortification vehicle involves considerable technical difficulties. Iron(II) salts and powdered iron both discolour salt, particularly the cruder cooking varieties (92, 94), and the discoloration process is accelerated in hot humid environments. Deterioration in bioavailability has also been noted with the passage of time (Narasinha Rao, unpublished observations, 1974). Iron(III) orthophosphate has proved to be a more satisfactory form of iron for fortifying salt and has been reported to have reasonable absorbability provided ascorbic acid or sodium hydrogen sulfate (Narasinha Rao, unpublished observations, 1974) is also present.

(c) *Sugar*

In some countries sugar has many of the necessary characteristics for a fortification vehicle. It is widely consumed, processed in a small number of places, and is not discoloured by the addition of iron(II) salts. Unfortunately, this fortified sugar produces a blackish discoloration and precipitate in tea (85). The use of iron(III) orthophosphate instead of iron(II) sulfate overcomes this difficulty but renders the iron much less absorbable.

In many developing countries very little refined sugar is consumed, which severely limits its potential usefulness as a fortification vehicle.

*(d) Infant foods*

Most cereals and milk powders for infants are routinely fortified with various forms of iron. In addition, many contain ascorbic acid, a known enhancer of iron absorption. There is good evidence that fortified infant cereals can improve iron nutrition (134, 135). From studies in adults on the absorption of iron from various cereals (91, 93, 94), it would seem advisable for infant formulas to include both an absorbable iron salt and ascorbic acid with an iron:ascorbic acid ratio of at least 1 : 10.

*(e) Fish sauce*

A fish sauce that is widely used in Thailand has been fortified with iron salts of ethylenediaminetetraacetate (EDTA), with evidence of benefit (136).

*(f) Skimmed milk*

Skimmed milk fed to preschool children has been fortified with iron(III) glycerophosphate. Since this iron is as readily absorbed as iron(II) sulfate, the potential usefulness of this vehicle is clear (137).

*5.1.3 Absorption promoters*

When salt fortified with 35–100 mg of ascorbic acid is used in the preparation of maize porridge or rice, the absorption of the intrinsic cereal iron is increased 2–4-fold (92, 94). Such fortified salt also enhances the absorption of iron added to the meal and may have a similar effect on the extrinsic contaminating iron present in many foodstuffs. If this is confirmed to be so, then a significant improvement in iron nutrition might be achieved in some communities by simply adding ascorbic acid to the diet in a vehicle such as salt or sugar.

*5.1.4 Predicting the effectiveness of fortification*

Any iron compound to be used for fortification must be tested for its availability by means of absorption tests using radioactively labelled material. Since there may be species differences in the ability to absorb different iron compounds, it is essential to conduct absorption tests directly in man rather than try to extrapolate the results obtained with experimental animals to man. Dietary iron absorption is related to iron status, which means that absorption of the test compound must be

compared with the absorption of a test dose of radioactive iron(II) ascorbate in the same individual. It is also known that absorbability of an iron product may vary from batch to batch and may alter with storage. These factors must likewise be taken into account.

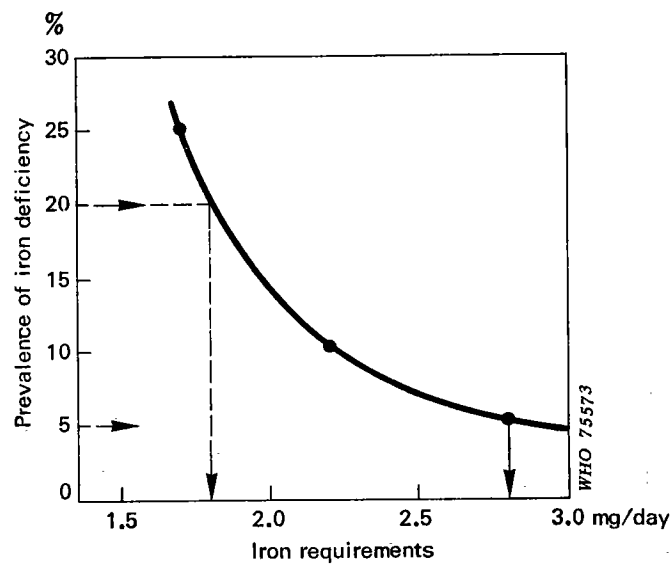
Having investigated the absorbability of an iron compound given alone, one must test the effect of adding increasing amounts of it to food. Studies on the absorption of iron salts given alone show that as the amount of iron administered is increased there is an increase in the amount absorbed but a decrease in the percentage absorption (73). However, when an easily soluble iron salt (e.g., iron(II) sulfate) is added to a meal, the situation is more complex. In one study it was found that the percentage absorption from whole meals did not decrease as the amount of iron added was increased up to 9 mg (137a). The increase in iron absorption that will be obtained by a given level of fortification is therefore difficult or impossible to predict in the absence of isotopic studies. If the iron product is one that exchanges well with the non-haem iron pool, these studies can be carried out (in diets without significant haem iron) by means of a single isotopic label. If, however, the product does not fully exchange with the non-haem iron pool, then its relative availability must be determined with the use of 2 isotopic labels, one to label the iron product and the other the non-haem food iron. Such studies will enable the total iron absorption, and hence the increase in iron absorption from the fortified diet, to be quantified and compared with the absorption of a reference dose of iron(II) ascorbate. Knowledge of the extra amount of iron absorbed will then permit an estimate to be made of the benefit to the iron deficient population (Fig. 3).

## 5.2 Folate

The fortification of foods with folic acid has only recently been explored. Many of the same considerations as apply to iron fortification apply to fortification with folate. In particular, attention must be paid to the effects of food processing, cooking, and storage on the folate content.

In a simulated fortification trial in pregnant women, the enrichment of a maize meal to provide a daily additional amount of 1 mg of folic acid was found to produce a significant rise in serum and red cell folate concentration and to prevent the development of anaemia, which was highly prevalent in the control group (22). Further studies showed that fortified maize and rice produced equal increments in serum folate

FIG. 3. PREDICTION OF THE EFFECTS OF FORTIFICATION ON THE PREVALENCE OF IRON DEFICIENCY



The effects of fortification can be predicted from the known distribution of iron requirements in women and the measured increases in iron absorption from typical meals at different levels of fortification. The increase in absorption needed to achieve a certain effect can also be calculated. Thus, if the mean absorption of iron is 1.8 mg/day the prevalence of iron deficiency will be 20%. Increasing this absorption by 1 mg/day will reduce the prevalence of the deficiency to 5% of the population.

concentration, which were about half as large as those obtained by the ingestion of a corresponding amount of a folic acid solution. However, enriched bread produced a smaller increment. The folate resisted destruction by boiling and baking at the temperatures and for the cooking periods used for the conventional preparation of such foods. Baking for longer periods, however, resulted in the loss of folate activity (N. Colman et al., unpublished observations, 1974). In another study, folate-fortified maize meal containing enough folate to result in a daily additional consumption of 300–500  $\mu\text{g}$  was given to 5 lactating women with megaloblastic anaemia due to folate deficiency. All of them showed optimal haematological responses and there was no secondary reticulocyte response in 2 of the patients who were subsequently given pharmacological doses of folic acid (138). On the basis of these observations, it would be justified to investigate further the feasibility of folate fortification in population groups where there is a high prevalence of folate deficiency.

## 6. A SCHEME OF ACTION FOR COMBATING NUTRITIONAL ANAEMIA

In order to assist public health authorities in planning appropriate action to combat nutritional anaemia, the following plans of action were outlined at the meeting. They are summarized in Fig. 4, pages 32-33.

### 6.1 Definition of the problem (Fig. 4.1)

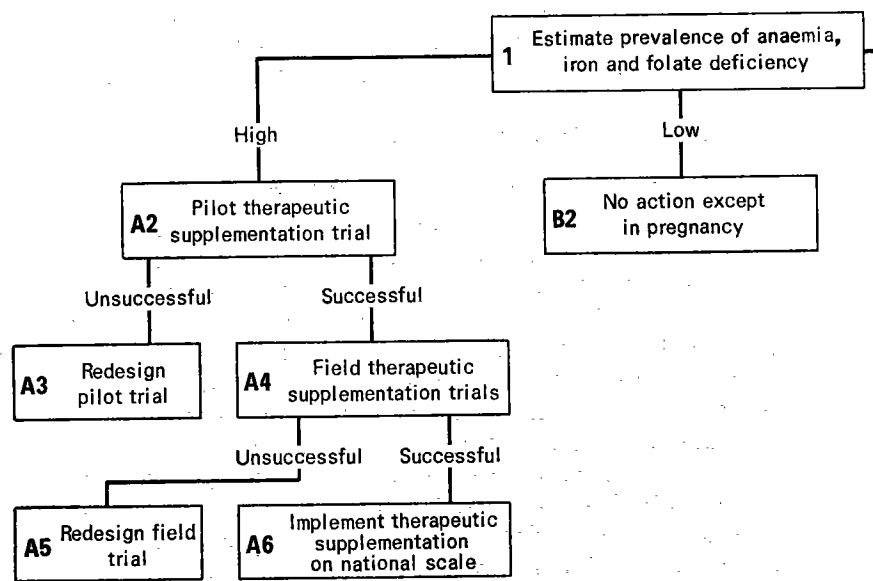
#### 6.1.1 *Determining the status of the population*

In many countries information already exists on dietary intakes and on the prevalence of nutritional anaemia and deficiencies of haematopoietic nutrients. However, in other countries this information is still lacking and should be obtained. The procedure adopted for selecting the population to be studied is of fundamental importance and often receives too little attention. Ideally, the group studied should be a statistically selected random sample of the whole population (76, 139). Where this is not immediately feasible, studies should be done in those population groups most likely to be at risk, namely, preschool children and women of childbearing age. Since the demand for haematopoietic nutrients is greatest during pregnancy, existing deficiencies become more manifest and can be most readily detected at this time. The status of pregnant women is therefore a sensitive index of the situation in those segments of the community from which the pregnant women are drawn. Measurements of haemoglobin concentration and/or packed cell volume can provide information on the frequency distribution of these values and make it possible to estimate the prevalence of anaemia (76). At the same time, wherever possible, measurements should also be made of serum iron, percentage saturation of transferrin, serum ferritin, serum and red cell folate, total serum proteins, and serum albumin. These measurements will permit a more precise determination of the nutritional status of the population and will also yield valuable information about the nature of the deficiencies responsible for the anaemia. However, if the prevalence of anaemia is high and facilities for all these determinations are not available, public health action can still be initiated on the basis of determinations of haemoglobin and/or packed cell volume alone.

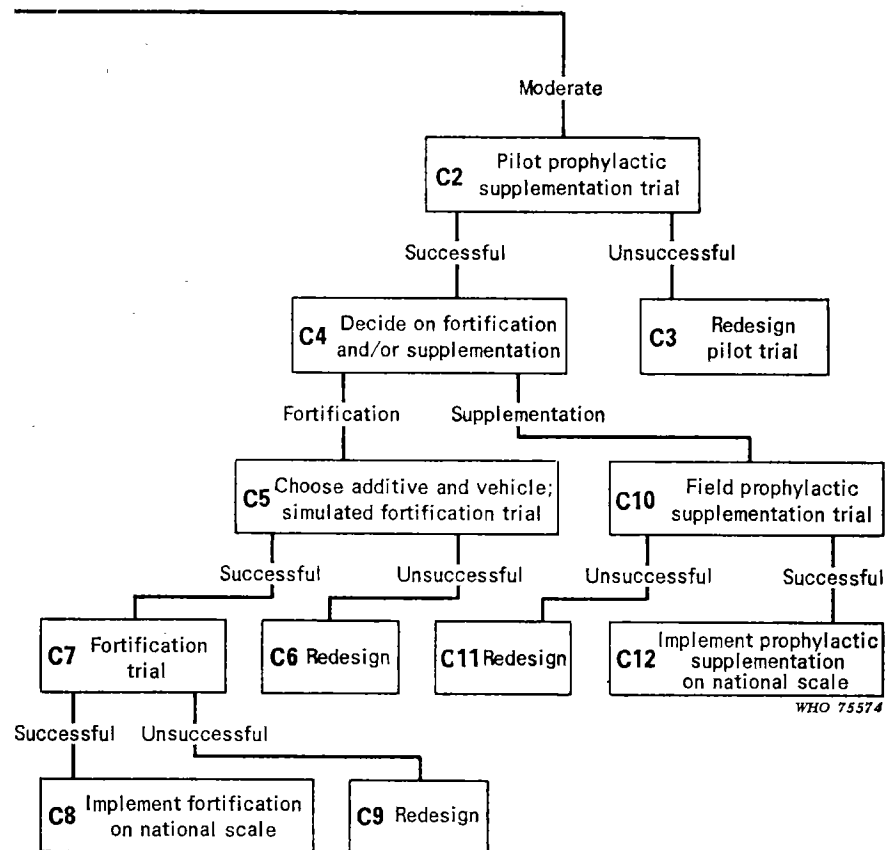
#### 6.1.2 *Setting the goals*

Every individual has a homeostatic mechanism which, in times of health and in the presence of optimum supplies of nutrients, sets the haemoglobin concentration at a level that can be regarded as normal for

FIG. 4. SCHEME OF ACTION FOR THE CONTROL







him. A frequency curve of such normal haemoglobin concentrations approaches a Gaussian distribution (140). The frequency distribution curve for the normal haemoglobin concentrations of a given population can be determined only by first excluding deficiency states, either by means of specific laboratory determinations (141) or by prior administration of haematinic supplements (142). Such frequency distribution curves will probably be found to be identical in different parts of the world, after due allowance has been made for age, sex, pregnancy, and altitude of residence. It has been suggested that a haemoglobin concentration of less than 13 g/100 ml of blood in adult males, 12 g/100 ml in non-pregnant women of childbearing age, and 11 g/100 ml in pregnancy is likely to indicate anaemia (2). This is a useful practical guideline, but it is an oversimplification. For example, even in a normal healthy population a small proportion of subjects will have a haemoglobin concentration below these values, whereas some individuals with a higher haemoglobin concentration may be anaemic and show an increase in haemoglobin following therapy. A more scientific approach is to determine the proportion of the population likely to be suffering from anaemia at any given haemoglobin concentration by comparing the observed frequency distribution curve with the frequency distribution curve of normal haemoglobin concentrations (13, 142). Whichever approach is used, minimal acceptable standards should be laid down in each country. These goals may be expressed in terms of either the acceptable proportion of individuals having a haemoglobin concentration below a certain level, or the acceptable probability of an individual being anaemic at a given haemoglobin concentration. The higher the standards are set the more difficult and expensive they will be to achieve. In setting them one should therefore take account of the financial and manpower resources available and the other health needs of the country.

If the prevalence of anaemia and nutritional deficiency is low no public health action is needed except for pregnant women, who may require prophylactic supplementation to meet the extra demand for nutrients (Fig. 4.B2). In areas of higher prevalence, however, action programmes may be needed to deal with the situation. Such areas can be considered in two broad categories, namely, those with a high prevalence of nutritional anaemia and those with a moderate prevalence.

## **6.2 Areas with a high prevalence of anaemia**

Having determined that a particular population has an unacceptably high prevalence of anaemia, one should initiate a programme of therapeutic supplementation as outlined below.

#### 6.2.1 *Pilot therapeutic supplementation trial* (Fig. 4.A2)

The precise design of this trial may differ from region to region and will depend to some extent on the information already available on dietary patterns and the nutritional status of the population as regards iron, folate, and vitamin B<sub>12</sub>. From the public health standpoint nothing indicates that vitamin B<sub>12</sub> deficiency plays a significant role in the causation of nutritional anaemia, even in countries where the intake of this vitamin is very low, such as India (16, 21). Therefore, unless there is good local evidence to the contrary, vitamin B<sub>12</sub> deficiency may probably be ignored. If folate nutrition has been shown to be adequate, the question to be addressed by the pilot trial is how to deal with iron deficiency (see section (a) below). If both iron and folate deficiencies exist, or if it has not been possible to determine with reasonable certainty the nature of the deficiencies, the pilot trial must focus on both iron and folate (section (b)). Rarely, iron nutrition may be normal and the problem may be that of folate deficiency alone (section (c)).

##### *(a) Pilot therapeutic iron supplementation*

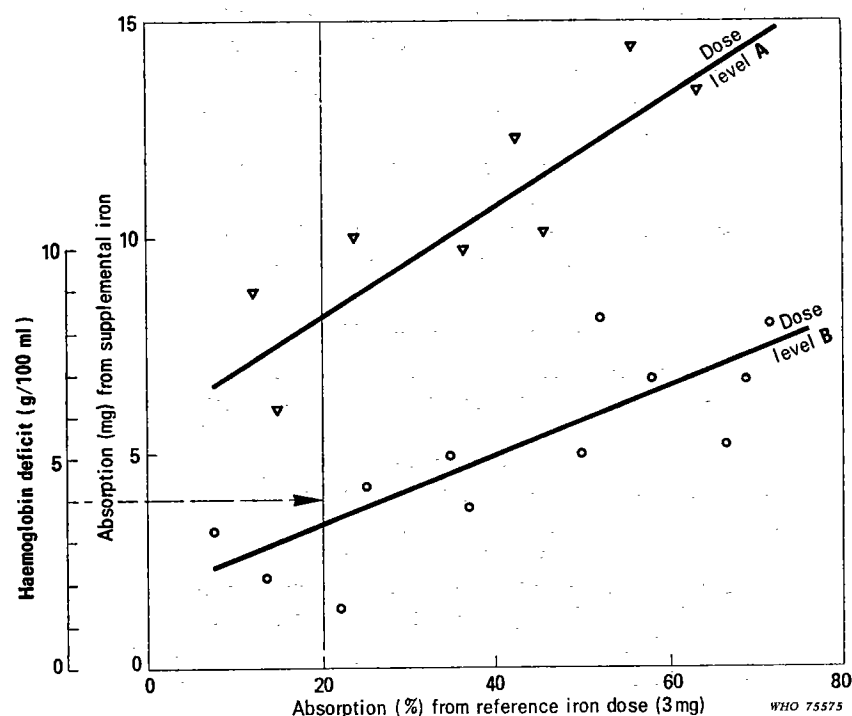
In populations in which iron deficiency is known to be the cause of the anaemia the pilot therapeutic trial should be carried out with iron supplements.

Absorption of supplemental iron is influenced by a number of factors besides the iron status of the subjects, such as the composition of the diet and the prevalence of various disorders of the gut. This is why it is necessary to estimate by means of a pilot trial what dosage of a given form of iron will be needed to achieve a specified effect in the target population within a given period of time, e.g., 3 months. Such an estimate can be made in two ways: (i) by measuring absorption with radioactive isotopes, and (ii) by studying the haemoglobin response to test dosages.

Where appropriate facilities are available, absorption from radioactive labelled iron tablets, at 2 or more dose levels in the range of 50–200 mg, should be studied in a sample of non-pregnant subjects from the population. The subjects need not necessarily be selected at random but should cover the whole spectrum of iron status, including iron deficiency anaemia. The labelled iron tablets used for the trial should be similar in all other respects to those to be used subsequently for supplementation and should be given in the same way (e.g., with the same meal) as is planned for the actual supplementation programme. The tablets should be given for a number of days in order to minimize

the effects of day-to-day variations in absorption. In each subject the absorption from a reference dose of 3 mg of iron as iron(II) ascorbate, given on a fasting stomach, should also be measured. The absorption from the supplemental iron tablets is then plotted against the absorption from the reference dose for each subject (Fig. 5) and the line of best fit

FIG. 5. ESTIMATION OF DAILY DOSE OF IRON NEEDED TO CORRECT HAEMOGLOBIN DEFICIT \*



\* See explanation in text.

for each dose level is drawn. It is known that iron absorption decreases during iron repletion. In subjects without iron deficiency the average absorption from a reference dose is about 20%. Therefore, the intercept of the lines of best fit with a vertical line at 20% absorption of the reference dose (Fig. 5) will indicate approximately the average amount of iron that can be expected to be absorbed from the iron tablets at each dose level after prolonged supplementation, when the absorption capacity has dropped. If it is planned to achieve a normal haemoglobin concentration

within a period of 3 months, experience has shown that approximately 1 mg of iron must be absorbed a day for every g/100 ml deficit in haemoglobin. (This assumes that iron losses are normal and does not allow for depleted iron stores.) The daily dose that would be required to ensure the absorption of this amount can then be taken from the figure by interpolation between the intercepts of the two dose levels (see Fig. 5, dashed line).

When isotopic studies are not feasible, or when it is desired to confirm such studies, a pilot trial can be carried out to study haemoglobin responses in groups of subjects receiving different doses of supplement.

The trial subjects must be a representative sample of the population. Selection from a special section of the community, e.g., patients contacting or being treated in a hospital, cannot necessarily be assumed to provide such a sample. There must be one group for each dose level to be tested, plus a control group given only a placebo. The practice of using non-anaemic subjects as the "control" group is not acceptable because it is important that the composition of this group should be the same in all respects as that of the other groups. The trial subjects must be allocated randomly to the various groups by means of an appropriate random table. In populations where there is a very high prevalence of anaemia it may be wise first to divide subjects into several strata according to their initial haemoglobin concentration and then, within each stratum, to allocate subjects randomly to the various treatment groups (Fig. 6). This will help to ensure a more even composition of the groups in terms of initial haemoglobin concentration.

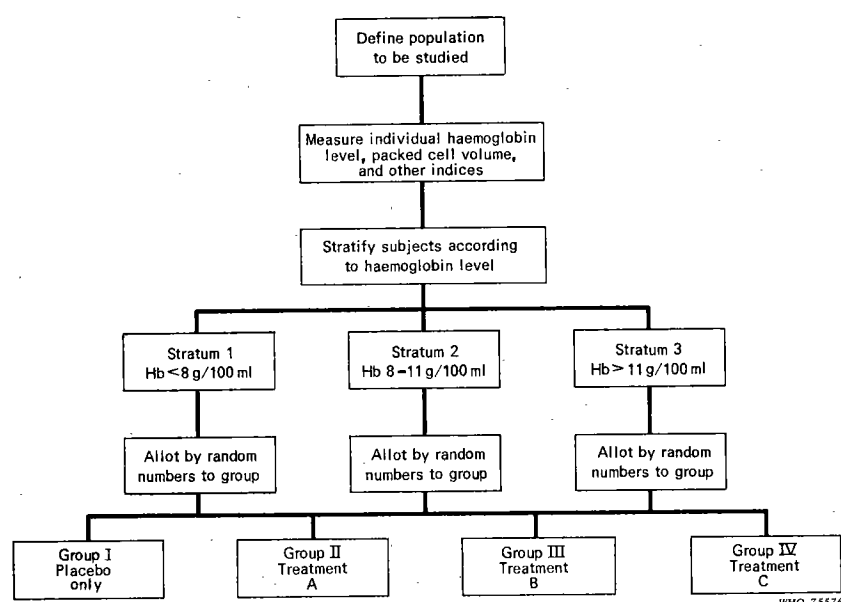
In order to prevent subjects in any group from suffering ill effects from their anaemia, very severely anaemic individuals with a haemoglobin concentration below a predetermined level should not be included in the trial, and any person whose haemoglobin drops below this level in the course of the trial should be excluded.

The number of subjects needed in each group will depend on the severity of the anaemia and on the degree of certainty with which it is desired to detect a given rise in haemoglobin. In practice, a minimum of at least 30 subjects completing the trial will be necessary in each group.

A number of factors should be taken into consideration when deciding how long the trial should last. In general the longer the trial the more marked the effect of small doses of supplement. However, it is probably most economical if the design of the initial pilot trial calls for fairly large doses given over a relatively short period of time.

It is essential that the daily taking of the tablets should be supervised by a person entrusted specifically with this responsibility. Unless

FIG. 6. DESIGN OF A SUPPLEMENTATION TRIAL IN A POPULATION WITH A HIGH PREVALENCE OF ANAEMIA TO TEST THE EFFECT OF A NUTRIENT AT 3 DOSE LEVELS



consumption of the supplement is ensured by personal supervision, erroneous conclusions may be drawn from the results.

The results of the trial can be analysed in a number of ways, for example, by determining the mean haemoglobin rise in each group or the number of anaemic individuals remaining in each group. Since the lower the initial haemoglobin level the greater the expected response, a sensitive method of analysis is to calculate, for each group, the regression function of final on initial haemoglobin concentration and then compare the regression functions of different groups (Tables 3 and 4, pages 22 and 24).

(b) *Pilot therapeutic iron and folate supplementation*

When anaemia of high prevalence is known to be due to combined iron and folate deficiency, or when the relative roles of iron and folate deficiency are not clear, the pilot trial should include both these supplements. Where possible, an estimate of the daily dose of iron required may be obtained from isotopic studies as outlined on pages 35-37 (Fig. 5). If isotopic studies are not feasible 2 dose levels of iron can be

arbitrarily chosen, for example, either 50 and 100 mg of iron in populations with a diet high in animal protein or 100 and 200 mg of iron in populations with a diet low in animal protein. The dose of folate required will probably be in the range of 500–1000 µg, although larger doses such as 2 mg will give a greater margin of safety, especially in pregnant women.

The number of groups needed will depend on how many dose levels have been selected for the supplements to be tested. In the simplest type of trial one dose level of iron and one of folate are tested with 4 groups that are given, respectively, placebo, iron alone, folate alone, and both iron and folate. Additional dose levels obviously require more groups (see, e.g., pages 19–21). The procedures for allocating subjects to the groups and for interpreting the results are described in section (a) above.

*(c) Pilot therapeutic folate supplementation*

In the rare cases in which iron nutrition is normal and folate deficiency alone is responsible for the prevalent anaemia, the pilot therapeutic trial can be conducted by studying the haemoglobin response to different doses of folate in appropriately chosen groups, as described above.

*(d) Outcome of pilot therapeutic supplementation*

Analysis of the results of the pilot therapeutic trial may reveal either an inadequate response or a satisfactory one. If the response was unsatisfactory either inadequate doses of supplement were used or they were poorly absorbed, or alternatively the anaemia was due to a deficiency of nutrients not included in the trial. In such a case, the trial must be redesigned (Fig. 4.A3) and the study repeated.

If the analysis discloses that the response was satisfactory then it may be desirable to conduct a further trial to determine the lower limit of effective supplementation. It should, however, be borne in mind that the major cost of a supplementation programme is not the amount of active substance in the tablets but the tablets themselves and in particular the administrative and other costs involved in their distribution.

*6.2.2 Field therapeutic supplementation trials (Fig. 4.A4)*

In public health practice it is impossible to supervise the daily consumption of supplements. Pilot trials must therefore be followed by trials under field conditions before any programme can be recommended for implementation on a national scale.

Field trials should be conducted on the population groups at greatest

risk. Their precise design will depend on the nature of the health services infrastructure of the country concerned. For example, where antenatal clinics or visiting midwives provide good coverage of pregnant women, they can be used for distributing tablets to the women. Along with tablet distribution a certain amount of health education is necessary to encourage subjects to take their tablets regularly. In deciding on the dose of supplement to be used one should take into consideration the fact that tablet consumption will often be irregular.

Subjects should be divided randomly (with stratification if there is a very high prevalence of anaemia) into 2 groups, one receiving the trial supplement and one receiving a placebo of identical appearance. The time interval between successive distributions of tablets should be the same as the interval anticipated when the programme is applied on a national scale. This interval should be as short as possible, depending on the number of visits the health worker can make to the subjects or the number of visits the latter can be persuaded to make to the local health centre or clinic. Clearly, the more frequent their contacts with the health worker, the more likely are subjects to take their supplement. On the other hand, the greater the frequency of the contacts, the more health personnel will be needed and hence the greater the cost of the supplementation programme. In practice, a balance must be struck between these two factors. However, there is probably a certain minimum of contact necessary, depending on the comprehension and motivation of both the subjects and the health workers, below which no useful results can be expected. It should also be remembered that iron tablets represent a potential hazard to young children if accidentally ingested in large numbers, and the less frequent the contacts the more tablets the subject is likely to have at home at one time.

At the end of the trial period the results may be assessed by the same methods as were used for analysing the pilot supplementation trial.

If the field trial fails to reduce the prevalence of anaemia to an acceptable level, the trial should be repeated with one or more of the following modifications :

- attempting to increase the regularity of tablet consumption by distributing tablets more frequently ;
- enhancing the understanding and motivation of the health workers involved in the trial and intensifying health education to impress on subjects the importance of regular tablet consumption ;
- raising the dose of supplement by increasing either the amount in one tablet or the number of tablets to be taken each day.



If in spite of various modifications the field trials still fail to produce adequate results, it may be desirable for the available health workers to concentrate their efforts on the more anaemic subjects who are the most likely to suffer ill effects or even death from their condition. This presupposes some simple method by which health workers can determine the more severely anaemic individuals (for example, those with a haemoglobin concentration below 8 g/100 ml) with a reasonable degree of certainty. The practicability of this kind of screening needs to be investigated. If such individuals can be identified, then supplementation trials involving more frequent visits by the available health workers could be designed to cover those subjects alone.

#### 6.2.3 *National therapeutic supplementation programme* (Fig. 4.A6)

After field supplementation trials have been successfully carried out in population groups at highest risk, a national programme of supplementation to cover these groups can be designed, costed, and initiated. Once in progress the programme should constantly be monitored for effectiveness by periodic checks of haemoglobin concentration and/or packed cell volume in randomly selected samples of individuals covered by the programme.

Since therapeutic supplementation is, by definition, aimed at treating anaemia within a reasonably short period of time, a successful programme will result in a population with a lower prevalence of anaemia for whom (with the exception of pregnant women) supplementation at therapeutic levels may no longer be necessary. Wherever possible, steps should be taken to provide continuing prophylactic measures in the form of fortification or low-level supplementation (see following section) to ensure that the treated population continues to have a low prevalence of anaemia.

### 6.3 **Areas with a moderate prevalence of anaemia**

In areas where anaemia is only moderately prevalent, measurements of, e.g., serum iron, percentage saturation of transferrin, red cell protoporphyrin, serum ferritin, and serum and red cell folate are essential to determine the prevalence of iron and folate deficiencies. If there is a significant prevalence of either of these deficiencies an attempt should be made to increase intake of the deficient nutrient. Since it is very difficult to change existing food habits, food fortification may be the best approach to increasing dietary intake. Fortification

programmes may be directed either at whole communities, if the prevalence of the deficiency is high throughout the community, or at vulnerable population groups that can be reached through particular foods — for example, infants, who in some countries can be reached by fortification of processed baby foods. In certain population groups where fortification is not practicable, long-term low-level supplementation may be necessary.

#### 6.3.1 *Pilot prophylactic supplementation trial* (Fig. 4.C2)

Before any food fortification or low-level supplementation programme is embarked upon, a pilot supplementation trial should be carried out to determine the effectiveness of a given supplement in reducing the prevalence of the deficiency. If only one nutrient is involved the trial should be designed so that one control group receives a placebo and several groups receive increasing amounts of the nutrient in question. In order to mimic subsequent fortification procedures as nearly as possible the supplement should be consumed with meals. Close supervision of the subjects is essential to ensure regular consumption of the supplement.

In the case of iron, the amount absorbed from the diet can be measured by preliminary radioisotopic studies using the extrinsic tag technique. This will make possible a fairly precise estimate of the quantity of extra iron that must be absorbed to improve the iron balance by a given amount (Fig. 3, page 30). Further isotopic studies can then be carried out to determine how much iron must be added to the diet to ensure the absorption of that quantity. If isotopic methods are not used, the results of the trial can be assessed only by determining whether the prevalence of the deficiency, as measured by the appropriate parameters, has decreased to an acceptable level while the prevalence in the control group has remained unchanged.

If the pilot trial fails to give satisfactory results it will have to be redesigned (Fig. 4.C3) to include either larger supplements or, in the case of iron, supplements given on a fasting stomach to avoid the inhibiting effects of food.

#### 6.3.2 *Fortification or supplementation?* (Fig. 4.C4)

From the results of the pilot trial a decision must be made whether the prophylactic programme can best be carried out by fortification or by supplementation. Particularly in the case of iron, the large amounts that must be absorbed and the poor absorbability from many diets may require the addition of so much extra iron as to render fortification

clearly impossible. Further, the dietary habits of the population may be such that there is no suitable vehicle available for fortification. In the face of either of these situations, attempts at prophylaxis should proceed by way of supplementation. Otherwise, the possibility of fortification should be explored since it offers an opportunity of supplying a continuous food source of iron to a population.

#### 6.3.3 *Choice of additive and vehicle for fortification ; simulated fortification trial (Fig. 4.C5)*

The choice of a suitable form of the nutrient for fortification should involve a consideration of its availability for absorption, its stability on storage and cooking, and its effects on the food. The vehicle to be suitable must have the following characteristics : it must be compatible with the nutrient ; it must be widely consumed in adequate amounts ; and it must be centrally processed (or available) in a small number of centres to permit adequate overseeing and quality control of the fortification.

Many of the required characteristics of both additive and vehicle for iron fortification are discussed in detail on pages 25-28 of this report.

Once the form of the nutrient and the vehicle have been determined, a simulated fortification trial should be conducted. In simulated trials the nutrient is added to the meal in the amount and at the point at which it will be added once it is actually combined with the vehicle. For example, if it is planned to fortify common salt with iron, in the simulated trial the iron product can be added to the uncooked food in appropriate amounts, depending on the proposed level of fortification. In the case of iron, initial radioisotopic studies should be carried out to determine how well the labelled iron compound is absorbed. If these studies show adequate absorption, and if the fortified product is already available, it may be possible to proceed directly to a fortification trial (section 6.3.4).

However, in order to be certain that prolonged iron fortification at this level will have the desired effect, it may be wise to conduct a longer trial with unlabelled iron in place of or as confirmation of the isotopic studies. The trial should involve 2 groups of subjects, one acting as a control and the other receiving the same food to which the extra nutrient has been added. In the case of folate, for which radioisotopic techniques are not satisfactory, such an approach is the only one possible. The ideal centre for the trial would be a residential institution where strict dietary control and random allocation to groups are possible. Since relatively small amounts of nutrient are involved, assessment of results

may be feasible only after some months depending on the size of the supplement and the degree of deficiency (in the case of folate 1–3 months and in the case of iron 6 months or more). If the trial is unsuccessful it must be redesigned (Fig. 4.C6) and repeated, or alternatively a decision must be made that fortification will not work and that prophylactic supplementation will be needed (see section 6.3.6).

#### 6.3.4 *Fortification trial* (Fig. 4.C7)

When a simulated fortification trial has been demonstrated to be successful, a fortification trial proper should be carried out. The fortified food product is made available to one group of subjects while a comparable control group continues to receive the traditional product. This trial may also be performed initially in a residential institution but it should subsequently be repeated under more realistic conditions, for example, where the product is made available through the usual supply channels to all the families in one village but not to a similar village, which acts as a control.

Results should be assessed by determining whether there has been an appropriate improvement in the nutritional status of those receiving the fortified product as compared with the control group.

#### 6.3.5 *National fortification programme* (Fig. 4.C8)

Once fortification trials are shown to be successful a national fortification programme can be planned, costed, and implemented. However, the effectiveness of such a programme must be continually monitored to ensure that the added nutrient continues to be readily available (this is particularly so in the case of iron salts where there may be marked, inexplicable batch-to-batch variation) and to confirm that with changing dietary patterns the level of fortification remains adequate.

#### 6.3.6 *Field prophylactic supplementation trial* (Fig. 4.C10)

In situations where food fortification is not practicable, the only possible approach to prophylaxis is supplementation. The pilot prophylactic supplementation trial (Fig. 4.C2) will already have indicated the probable amounts of supplement needed, and a field trial can be conducted in the same way as the field therapeutic supplementation trial (see page 39), the only difference being the lower level of supplementation. Because of the lower prevalence of anaemia, assessment of the results cannot depend only on haemoglobin and/or packed cell volume measure-

ments but must include more direct measurements of iron and/or folate nutrition.

If the field trial is not successful it will need to be redesigned (Fig. 4.C11) and repeated.

#### 6.3.7 *National prophylactic supplementation programme* (Fig. 4.C12)

After the field prophylactic supplementation trial has proved successful, a prophylactic supplementation programme can be initiated on a national scale. As with other types of national programme constant monitoring will be necessary to ensure its continuing effectiveness.

## 7. RECOMMENDATIONS

Surveys sponsored by WHO and other agencies have revealed a high prevalence of nutritional anaemia, especially iron deficiency anaemia, in many countries. While much fundamental information is still lacking, the causes, implications, and public health significance of nutritional anaemia are sufficiently well established to justify a series of recommendations dealing with information needs in this area and with action for control and eradication.

### 7.1 Methodology

(a) The WHO iron reference centre in Seattle, WA, USA, has performed valuable services as regards the standardization of serum iron estimations and the supply of standards and unknown samples to investigators collaborating in WHO projects. The standardization of measurements of total iron-binding capacity has not been so successful and requires further study. The services of this reference centre should continue to be available.

(b) The newly developed radioimmunoassay of serum ferritin appears to be an excellent method of assessing iron stores and may be of particular value for determining changes in iron balance brought about by supplementation or fortification. Because of the complexity of this assay, it might be useful to establish a central collaborating laboratory to which investigators participating in WHO-sponsored studies could send their specimens. In addition, however, antigen, labelled antibody, and appropriate standards should if possible be provided to selected laboratories wishing to establish the method.

(c) To permit the results of assays in different collaborating laboratories to be more validly compared, interlaboratory standardization of the measurement of serum and red cell folate concentrations should be improved and a more standardized method for the quantification of dietary folate should be developed.

(d) In some areas, there is a need for a simple screening method with which public health workers can detect severely anaemic individuals (e.g., those with a haemoglobin concentration below 8 g/100 ml of blood). Field trials of such methods will have to be conducted to determine their reliability.

## **7.2 Detrimental effects of nutritional anaemia and nutritional deficiencies**

The detrimental effects of mild anaemia, and of iron and folate deficiency without anaemia, must be more precisely defined. Once these are known, it will be possible to make realistic cost/benefit analyses of proposed control programmes.

(a) Recent studies have shown that anaemia reduces maximum or near-maximum work performance. It is imperative that these investigations should be expanded to include studies of work output and productivity under field conditions, with careful attention being given to experimental design and method. To facilitate the design of appropriate studies the investigators involved should be brought together with experts in work output determinations, anthropologists, biostatisticians, and health economists.

(b) While some evidence already exists that iron deficiency may affect both cellular and humoral immunity and alter granulocyte function, further studies are required on the effects of iron deficiency apart from its influence on haemoglobin synthesis.

(c) The deleterious effects of folate deficiency in the absence of anaemia need to be determined.

## **7.3 Availability of iron from different diets**

It has long been known that the amount of dietary iron available for absorption is extremely limited, but only recently have accurate isotopic methods become available with which absorption can be quantified. Studies done with these methods have revealed major differences in the availability of iron from different diets. In the light of these differences, statements of the amount of iron ingested, expressed in mg/day and calculated from tables or chemical measurements, are of very limited value.

(a) Further radioisotopic studies should be conducted on the availability of iron in typical diets from different areas. They should include the measurement of the absorption of a reference dose of iron (3 mg, as iron(II) ascorbate) to permit comparisons between different diets and subjects. This information will make it possible, once the total iron content of the diet is known, to arrive at valid estimates of the amount of iron that both normal and iron deficient subjects can be expected to absorb from the diet. More rational design of programmes to eradicate iron deficiency will also become possible.

(b) Further information about inhibitors and promoters of iron absorption should be acquired and applied to efforts to increase the absorbability of dietary iron, especially in areas where iron deficiency is prevalent.

#### **7.4 Prevalence of nutritional anaemia and deficiency of haematopoietic nutrients**

In many countries adequate prevalence data are already available. Where this is not so, suitably planned and statistically controlled surveys should be carried out. Ideally, in addition to haemoglobin and/or packed cell volume, such surveys should measure serum iron concentration, percentage saturation of transferrin, and concentrations of serum ferritin, serum and red cell folate, and serum vitamin B<sub>12</sub>. However, even where only haemoglobin and/or packed cell volume are measured, the finding of a high prevalence of anaemia may still provide a basis for public health action.

#### **7.5 Supplementation**

In population groups where there is a high prevalence of nutritional anaemia pilot therapeutic supplementation trials should be undertaken. These trials should initially focus on people having the highest prevalence of anaemia and those most likely to suffer ill effects from anaemia, such as pregnant women and preschool children. It may also be useful to study certain defined and accessible population groups, e.g., school-children or workers in large industrial establishments.

(a) Where the nature of the deficiency (or deficiencies) responsible for the anaemia is not clear, this may need to be determined by a pilot therapeutic supplementation trial.

(b) When the nature of the deficiency is known a pilot supplementation trial must be carried out to determine the amount of supplement required to correct the deficiency.

(c) Field therapeutic supplementation trials should follow the successful completion of the pilot supplementation. These will also involve operational research into the most effective method of distributing supplements within the limits of the resources available.

In some situations it may be necessary to concentrate control efforts on the anaemic sector of the population. Operational studies are needed in various countries with a high prevalence of anaemia to test the practicability of this approach.

(d) Once adequate information is available from pilot and field supplementation trials, a national therapeutic supplementation programme should be instituted among those population groups at highest risk. Any such programme must be continually monitored to ensure that its effectiveness is maintained.

#### **7.6 Fortification with iron**

In a number of developed countries fortification of foods with iron is already practised. Many of these fortification programmes were started before certain investigational techniques now available had been developed. There is, therefore, only limited information as to the absorbability of the added iron and the effectiveness of the fortification.

(a) Further work is required to assess the availability of iron compounds currently used in fortification programmes (mainly in infant foods and flour and cereal products) and to evaluate the effects of these programmes.

(b) Where present fortification methods have failed to reduce the prevalence of iron deficiency to an acceptable level, existing programmes should be modified or new programmes devised.

(c) In any country where changes in the fortification programme are envisaged or a fortification programme is to be initiated, selected groups should be carefully monitored (e.g., by following changes in serum ferritin) to study the effects of the supplementation; in particular, attention should be paid to individuals with low iron stores to see that they improve. Special attention should also be paid to those with impaired regulation of iron absorption such as occurs in haemochromatosis and thalassaemia to ensure that the extra iron does not contribute to the development of iron overload.

(d) In areas with a high prevalence of iron deficiency, attempts should be made to find a suitable combination of iron preparation and vehicle for use in fortification trials.



(e) When iron compounds are being tested for use in fortification programmes one criterion of suitability should be a biological availability, as shown by isotopic studies in man, equivalent to at least 75% of that of iron(II) sulfate. The use of compounds of lower bioavailability is not recommended since their effects are likely to be less predictable.

(f) In areas where there is a high prevalence of iron deficiency, once an iron compound and a vehicle have been chosen simulated fortification trials should be carried out; when these are successfully completed, field fortification trials should be designed and conducted.

(g) After field trials have been shown to produce the desired results a national iron fortification programme should be planned and implemented, with adequate and continuous monitoring to ensure its success.

#### **7.7 Fortification with folate**

In populations with a high prevalence of folate deficiency, attempts should be made to find a suitable vehicle for folate fortification and appropriate trials carried out to determine the feasibility and effectiveness of fortification.

#### **7.8 Education and training**

The combating of nutritional anaemia will require trained personnel at all levels, from medical officers to public health workers, food technologists, chemists, and agriculturists.

(a) For those countries with insufficient specialized personnel to carry out these programmes, international assistance should be made available to help train national personnel, preferably in regional centres where conditions are most likely to approach those in the trainee's own country.

(b) It is recommended that several regional centres be established to assist in the above training programme and to act as reference and standardization centres for neighbouring countries.

#### **7.9 International cooperation**

Close international cooperation in solving the global problems of nutritional anaemia is highly desirable because it facilitates the exchange of ideas and experience and has a mutually stimulating effect on all concerned. In addition, institutions that are experienced in developing and applying the more complicated laboratory methods should be encouraged to assist research groups with more limited resources and capability.

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## Annex 2

**THE ABSORPTION OF IRON FROM WHOLE MEALS OF DIFFERENT TYPES AS MEASURED  
BY RADIOACTIVE IRON STUDIES**

TABLE 5. ABSORPTION OF IRON FROM TYPICAL VENEZUELAN DIETS \*

Subjects (n)	Geographic area	Chief ingredients of meals	Energy intake <sup>a</sup>	Intake of total iron (mg)	Absorption of non-haem iron (%)				Absorption of reference dose (%)
					Absorption of total iron (mg)	Breakfast	Lunch	Dinner	
15	Andes	Vegetables	1036 (4335)	10.4	0.4	1.5	2.4	2.0	14.1
12	Central	Vegetables	1119 (4682)	12.7	0.6	1.2	3.5	2.5	19.2
15	Coastal	Vegetables, fish, fruit	1351 (5653)	9.5	0.9	5.2	7.1	12.8	27.4
13	Coastal	Vegetables, fish, fruit	1351 (5653)	9.5	1.2	7.1	11.4	17.4	31.5
14	Andes	Vegetables	2000 (8368)	16.6	0.7	1.9	2.2	1.4	25.4

\* Data from M. Layrisse et al. (89) and from unpublished observations of N. Colman et al. (1974) and M. Layrisse (1974).

<sup>a</sup> Energy intake is expressed here in both kilocalories and kilojoules, the latter being shown in parentheses. Conversion factors are: 1 kcal = 4.184 kJ; 1 J = 0.239 cal.

TABLE 6. ABSORPTION OF IRON FROM INDIAN WHEAT DIETS \*

Subjects	Meal	Iron content (mg)			Absorption (%) (M $\pm$ SE <sup>a</sup> )
		Meal	Radioactive label	Total	
Normal (n = 17)	Wheat breakfast	5.1	1.0 <sup>b</sup>	6.1	2.1 $\pm$ 0.39
Hospitalized, non-anaemic (n = 5)	Wheat breakfast	5.1	1.0 <sup>b</sup>	6.1	1.5 $\pm$ 0.25
Normal (n = 10)	Wheat breakfast + wheat lunch	17.7	1.0 <sup>c</sup>	18.7	1.8 $\pm$ 0.34
Normal (n = 7)	Wheat breakfast + rice lunch	15.1	7.5 <sup>c</sup>	22.5	3.3 $\pm$ 1.01

\* Narasingha Rao, unpublished observations, 1974.

<sup>a</sup> Mean  $\pm$  standard error.<sup>b</sup> <sup>59</sup>FeCl<sub>3</sub>.<sup>c</sup> <sup>59</sup>FeSO<sub>4</sub>.

TABLE 7. ABSORPTION OF IRON FROM INDIAN RICE-BASED MEALS \*

Subjects	Meal	Iron content (mg)			Absorption (%) (M $\pm$ SE <sup>a</sup> )
		Meal	Radioactive label	Total	
Hospitalized, non-anaemic (n = 10)	Rice lunch	9.6	1.0 <sup>b</sup>	10.6	2.6 $\pm$ 0.87
Anaemic <sup>c</sup> (n = 7)	Rice lunch	9.6	1.0 <sup>b</sup>	10.6	12.6 $\pm$ 4.03
Normal (n = 60)	Rice lunch	9.6	5.0 <sup>d</sup>	14.6	5.5 $\pm$ 0.68
Hospitalized, non-anaemic (n = 7)	Rice-" Idli "	10.0	0.5 <sup>b</sup>	10.5	1.2 $\pm$ 0.42

\* Narasingha Rao, unpublished observations, 1974.

<sup>a</sup> Mean  $\pm$  standard error.<sup>b</sup> <sup>59</sup>FeCl<sub>3</sub>.<sup>c</sup> Haemoglobin concentration less than 10 g/100 ml of blood.<sup>d</sup> <sup>59</sup>FeSO<sub>4</sub>.

TABLE 8. ABSORPTION OF NON-HAEM IRON FROM SOME COMMON SWEDISH MEALS \*

Subjects (n)	Meal	Non-haem iron con- tent (mg)	Absorption		
			Diet (%) (M $\pm$ SE <sup>a</sup> )	Diet (mg)	Reference dose (%) (M $\pm$ SE <sup>a</sup> )
8	Meat, potatoes, beans, milk	3.1	11.6 $\pm$ 2.9	0.36	24.8 $\pm$ 2.6
	Sandwiches (cheese, sausage), milk	4.6	4.4 $\pm$ 1.4	0.20	
8	Pancakes, jam, milk	5.1	1.7 $\pm$ 0.4	0.09	20.4 $\pm$ 1.6
	Beans, pork, milk	5.4	4.0 $\pm$ 1.0	0.22	
9	Meatballs, potatoes, cranberries, milk	2.6	5.4 $\pm$ 1.3	0.14	19.5 $\pm$ 3.4
	Peasoup, pork, milk	3.5	4.8 $\pm$ 1.1	0.17	

\* L. Hallberg, unpublished observations, 1974.

<sup>a</sup> Mean  $\pm$  standard error.

IRON COMPOUNDS FOR USE IN IRON FORTIFICATION <sup>a</sup>

*Iron(II) sulfate*,  $\text{FeSO}_4$ , is widely used both for food fortification when soluble iron is considered necessary and in therapeutic doses for the treatment of acute iron deficiency anaemia. Two pharmaceutical forms of the compound are commercially available: dehydrated  $\text{FeSO}_4$  containing 1½–2 moles of water, and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; the water is loosely bound and migrates readily to dry-mix preparations, which makes the latter form unsuitable in many cases (143). Iron(II) sulfate tends to cause rancidity and discoloration in flour (144) and in high-protein food such as semisynthetic animal feed (14) and corn (maize)-soya-milk mixture (146). Nevertheless, it has been used as the iron compound in some weaning food preparations, e.g., the Kerala indigenous food (KIF) in India (147).

Feeding experiments with human volunteers using iron-fortified bread (148–152) or skimmed milk and sugar (146), with rats using bread (153) or infant formulas (154–156), and with weaned pigs (145) have confirmed that the bioavailability of either form is better than that of most other compounds tested. As regards cost, iron(II) sulfate at present is at the lower end of the price scale for comparable products. The toxicity level seems to be fairly high; the  $\text{LD}_{50}$  for rats is 500 mg of  $\text{FeSO}_4$ /kg of body weight (157). When fortifying common salt ( $\text{NaCl}$ ) with iron(II) sulfate, the National Institute of Nutrition, Hyderabad, India, recommends the preparation of a formula of containing, per kg of salt, 2500 mg of  $\text{FeSO}_4$ , 1000–2500 mg of  $(\text{NaPO}_3)_6$  (sodium hexametaphosphate) as complexing agent, and 500–1500 mg of  $\text{NaHSO}_4$  (sodium hydrogen sulfate) to avoid discoloration (158, 159). The additives do not influence the absorbability of the iron. In Guatemala it is planned to use sodium hexametaphosphate and disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) for the same purpose (160). Under moderate, rather dry climatic conditions even pure iron(II) sulfate added to salt and then cooked with rice is not detrimental to the colour and taste of the meal (161). Food processing seems to have little effect on the bioavailability of the compound (156, 162). The goal is to develop a “coated” or “stabilized” form of iron(II) sulfate that would inhibit the untoward effects on flour during storage but retain the good absorbability of this compound from the

<sup>a</sup> This annex is based on a document prepared by Dr Karin Schiele, Scientist, Nutrition, WHO, Geneva, Switzerland.

digestive tract (163). A USSR patent describes the mixing of iron(II) sulfate with some acids occurring in foods to ensure better availability (164).

*Reduced iron* can be produced by either hydrogen or electrolytic reduction of iron oxide; the former is used primarily in flour and corn (maize) enrichment (143). Its bioavailability and chemical reactivity depend on the particle size; the smaller the particles the better the absorbability (149, 165). In feeding experiments with human volunteers (148-151) and animals (145, 153, 166) the absorption of reduced iron baked into bread and rolls or given with high-protein food was the same (145, 148) or slightly lower (149, 151, 153) than that of iron(II) sulfate. However, in 2 cases (150, 166) several batches of elemental iron were found to be rather poorly absorbed. These 2 reports did not state the particle size of the iron used. When the ionizable iron content of a diluted hydrochloric acid extraction (simulated gastric juice) was taken as a standard to assess availability, the iron availability in white bread fortified with elemental iron exceeded that in wholemeal bread by approximately 24% (167).

The technological problems posed by fortification with reduced iron (rancidity of foods, etc.) are similar to those observed with iron(II) sulfate (168). According to experimental trials in Pakistan, however, reduced iron has fewer adverse effects on the quality of bread than do iron(II) sulfate and iron(II) gluconate (169). To avoid separation of the rather heavy iron particles from a cereal product upon shaking, a slurry of the iron could be applied together with an edible ester of glycerol or sorbitol and a fatty acid, as described in a recent US patent (170).

*Iron phosphates* are widely used by food manufacturers because they have little tendency to react with other food components. On the other hand, their bioavailability under certain conditions appears to be fairly low (148, 153, 155, 166). It can be increased considerably by heat treatment of the fortified food, e.g., by sterilization of a milk-based infant formula (156, 171) or by the baking of bread (153, 162) (both fortified with iron(III) orthophosphate or pyrophosphates). At first sight these findings seem to conflict with some of the above-mentioned experiments. In fact, however, they support the assumption that iron absorption depends on a large number of variables.

The following compounds have been used:

*Iron(III) pyrophosphate*,  $\text{Fe}(\text{P}_2\text{O}_7)_3$ , and *iron sodium pyrophosphate* are used primarily for the fortification of infant foods and pasta (143) as



well as in cocoa-containing products, where they do not readily produce any deterioration in colour (172) or flavour (173). Experimental studies have been conducted with bread fed to human volunteers (148, 162, 174) and animal feeding studies have been carried out with milk (175), bread (153), and a high-protein feed for weaned pigs (145). The results are conflicting.

*Iron(III) orthophosphate*,  $\text{FePO}_4$ , is used if an insoluble but not elemental form of iron is needed. Several states of hydration are known, the commercial products usually containing 3–4 moles of water. Their absorbability depends on the solubility in diluted acids; e.g., at stomach pH it may vary between 75% and 95% depending on the product (143). This fact might explain the conflicting evidence as to the bioavailability of the compound (152, 153, 155, 162, 166).

*Iron(III) polyphosphate*, a long-chained polyphosphate complex, precipitates protein from, e.g., industrial discharge such as commercial acid whey and potato waste. The lyophilized product contains 8–15% iron and 15–50% protein (176) and is white, fluffy, and mildly acid in flavour. It is thus of potential value for iron and protein enrichment of foods (177). When iron(III) polyphosphate is added to whole milk, 70–90% of the iron is found in acid precipitated casein in a non-dialysable form (178). This could be of value in cheese making. The bioavailability of an iron(III) polyphosphate powder seems to be comparable to that of iron(II) sulfate and iron(II) disodium ethylenediaminetetraacetate (145).

*Iron(III) sulfate*,  $\text{Fe}_2(\text{SO}_4)_3$ , has been tested as an additive to bread with human volunteers (148) and as a feed additive with anaemic chicks and rats (166). It has been found to be comparatively well utilized by the animals; the absorption rate from bread is approximately one-third of that of iron(II) sulfate.

*Iron(II) carbonate*,  $\text{FeCO}_3$ , is probably the most commonly used form of added iron in feeds for farm animals and poultry, although it did not prove very useful in feeding experiments with chicks and rats (166, 179).

*Iron(III) chloride*,  $\text{FeCl}_3$ , is used in the fortification of skimmed milk powder (180) and has also been tested with flour (181). Although some skimmed milks are susceptible to iron-catalysed oxidation, they are stabilized during the concentration which precedes drying and remain stable in both the powder and the reconstituted milk. To prevent polymerization during hydrolysis (iron polymers are biologically unavailable) the addition of 20 mmol of fructose, sorbitol, or xylitol or of citric or

gluconic acid (182) to 1 mmol of iron(III) chloride has proved useful. As regards bioavailability, iron(III) chloride seems to be at the higher end of the scale (166, 181).

*Ammonium iron(II) sulfate*,  $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4$ , was tested in animal experiments and proved to be fairly well absorbed (166).

*Iron(II) disodium ethylenediaminetetraacetate* and *iron(III) sodium ethylenediaminetetraacetate* deserve to be given special attention in the future. Experiments so far have shown an absorption rate similar to that of haemoglobin iron in both animals (145) and preschool children (160). They do not seem to enter the inorganic iron pool of the diet, i.e., their availability is not influenced by inhibiting substances in the food. Studies on the stability of iron(III) sodium ethylenediaminetetraacetate during cooking are planned at the Institute of Nutrition of Central America and Panama (INCAP), Guatemala, together with long-term field studies with fortified sugar (10 mg of iron from the compound/60 g of sugar) in larger population groups (160). Field studies in Thailand with fortified fish sauce (136) look promising. The US Food and Drug Administration has no objection to the daily administration of between 60 and 120 mg of this compound to adults but such individuals should be observed closely for deficiencies of trace elements (J. C. Chopra, personal communication to INCAP, 1974).

*Iron(II) citrate* was tested in India for salt fortification (158). The bioavailability was good as measured in male and female volunteers (absorption from salt only, 25% ; with food, 8%) but the stability of the fortified salt was insufficient. Upon storage at room temperature a yellowish-green colour developed within one week, most likely because of the moisture content of the salt. In rat feeding experiments with soya isolate the availability of iron(II) and iron(III) citrate was respectively 89% and 87% of that of iron(II) sulfate (156), whereas the intestinal absorption of a polymerized iron(II) tetrasodium dicitrate (molecular weight 1500) perfused *in situ* in rat duodenal tract was significantly lower than that of iron(II) sulfate (183). The authors suggest that the molecular weight of the chelates should not be too high.

*Ammonium iron(III) citrate* could become one of the most widely used iron compounds for food fortification. At present it is still comparatively expensive. It has been successfully added to flour (150, 153, 169, 184-186) as well as to dairy products (180, 187-189), where its undesirable side effects (colour deterioration with coffee, etc.) are less marked than those caused by most of the more soluble iron compounds

(172). Trials with cottage cheese seem to be particularly promising: 100 g of cottage cheese made from milk fortified with 20 mg of Fe/litre provides one-third of the recommended daily iron allowance for an adult female (188). For flour enrichment in regions with no cereal milling facilities, the use of a mixture of ammonium iron(III) citrate, riboflavin, and calcium together with dried yeast packed in separate plastic bags in one tin has been recommended and tested in Iran (186). In animal experiments the bioavailability of ammonium iron(III) citrate was comparable to that of iron(II) sulfate, fumarate, and others (166). Tests with 2 groups of 110–120 women each showed, however, that bread enriched with ammonium iron(III) citrate had no more influence on haemoglobin level than did ordinary bread (190).

*Iron(II) fumarate* might become the iron compound of the future, especially for blended foods (144). At present it is used in the USA for corn-soya-milk preparations (CSM) (450 mg of iron(II) fumarate/kg of CSM) for food assistance programmes (143) where iron(II) sulfate would cause rancidity. In feeding tests with young children 6% of the added iron was found to be absorbed (146). This means that a ration of 80–100 g of CSM/day containing 15 mg of added iron if given to children over 1 year old, in accordance with the WHO/UNICEF recommendation (146), would provide each child with 0.9 mg of extra iron a day. In the USA in 1970 the price of iron(II) fumarate although moderate was 7 times higher than that of reduced iron and approximately 3 times that of iron(II) sulfate (144).

*Iron(II) gluconate* is as yet probably too expensive to be considered for large-scale iron fortification programmes; in 1970 in the USA it was approximately 9 times the price of iron(II) sulfate (144). It is well absorbed by rats (156, 166, 191) and has no major adverse effects on the flavour of milk products (167). Some manufacturers use this compound for the fortification of certain infant foods so long as they do not contain cocoa (lest an adverse colour develop). An iron sorbitol-gluconic acid polymer has been successfully applied in the treatment of piglet anaemia (192). When added to flour in amounts exceeding 100 mg of Fe/kg of flour the compound seems to affect adversely the dough and bread quality (169).

*Iron(III) glycerophosphate* was chosen as the iron component for fortified milk in a programme to prevent protein-energy malnutrition in preschool children in Venezuela (193). There was no difference between the iron absorption from this compound and iron(II) sulfate. Similar results have been reported from animal experiments (156, 166).

*Iron(II) lactate* was extremely well absorbed by rats when mixed with soya isolates (156). It seems to be useful for milk fortification as well (173).

"*Iron fructose*", tested in a feeding experiment with guineapigs, was very well absorbed (194).

*Ferrocholine* (an iron-choline-citric acid complex) and *iron(II) tartrate* are both readily available forms of iron. Their absorbability has been tested in chicks and rats (166).

*Saccharated iron oxide* is regularly used by at least one of the important baby food manufacturers in Switzerland for iron enrichment of various kinds of milk-based foods for infants and young children (Dr H. R. Müller, Nestlé S.A., personal communication, 1974). A US patent (195) describes the preparation of a liquid iron-fortifying composition for nutritive purposes made of iron(II) or iron(III) compounds with corn syrup containing up to 40% sucrose.

In Hungary a method has been developed for the treatment of plant material containing more than 10% carbohydrates (wheat, rice, barley, potatoes, manioc roots, sugar cane) with iron(III) (196). The resulting complex, probably a mixture of iron-carbohydrate polymers of not exactly definable formula, is stable, tasteless, and biologically well utilized. It appears to be useful for both human and animal nutrition.

*Yeast*, because of its high iron content, might replace the above-mentioned fortifying agents in certain foods. Rye-wheat bread baked with 2% skimmed milk and 3% yeast had the greatest organoleptic and nutritive value out of a number of mixtures tested (197). A 500-g portion supplies 126% of the daily human iron requirement.

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